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(57) Abstract

The invention relates to methods for modulating the growth state of cells using secretin therapeutics and antagonists. The methods can be used as therapies for diseases caused by, or coincident with, aberrant glucose metabolism, such as Type II Diabetes Mellitus.

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Methods of Inducing Insulin Positive Progenitor Cells

This application is based on U.S. Provisional Application No. 60/119,575, filed February 10, 1999, incorporated herein by reference in its entirety.

Field of the Invention

The invention relates to therapies for treating glucose metabolic disorders (e.g., glucose intolerance, insulin resistance, hyperglycemia, hyperinsulinemia and Type II diabetes mellitus). The therapies are based on the discovery that secretin therapeutics modulate the growth state of insulin positive progenitor cells.

Background of the Invention

The endocrine portion of the pancreas is composed of the islets of Langerhans, which appear as rounded clusters of islet cells embedded within the exocrine pancreas. Four kinds of islet cells compose the endocrine portion of the pancreas: (1) alpha (α) cells, constituting 20% of islet cells, which secret glucagon, a hormone which raises blood sugar levels; (2) beta (β) cells, which secrete insulin, a hormone which lowers blood sugar levels; (3) delta (δ) cells, which secrete growth hormone inhibiting hormone (GHIH) or somatostatin, a hormone which inhibits the secretion of insulin and glucagon; and (4) ϕ cells, or pancreatic polypeptide (PP) cells, which synthesize pancreatic polypeptide. Glucagon acts on several tissues to make energy available in the intervals between feeding. In the liver, glucagon causes breakdown of glycogen and promotes gluconeogenesis from amino acid precursors. Pancreatic polypeptide inhibits pancreatic exocrine secretion of bicarbonate and enzymes, causes relaxation of the gall bladder, and decreases bile secretion. Insulin is known to cause the storage of excess nutrients arising during and shortly after feeding. The major target organs for insulin are the liver, muscle, and fat-organs specialized for storage of energy.

The most abundant cell in the islets, constituting 60-80% of the cells, is the insulin-producing β cell. Pancreatic development occurs in discrete stages and is regulated by endocrine hormones produced by pancreatic cells themselves or by other

tissues. In the rat, the pancreatic anlage forms at embryonic (e) day 10.15 (e10.5) by fusion of the dorsal and ventral pancreatic primordial buds that arise as protrusions from the duodenal endoderm. (Pictet, R. and Rutter, W.J. (1972) "Development of the Embryonic Endocrine Pancreas." In D. Steiner and N. Freinkel (eds.) *Handbook of Physiology*, The Endocrine Pancreas, Vol. 1, Section 8, Am. Physiol. Soc., pp. 25-66; Myrsén-Axcrona, U. et al. (1997) *Regulatory Peptides* 68:165-75.). Islet hormones appear sequentially in the developing pancreas: for example, glucagon appears at e10 in mouse and e11 in rat, insulin producing cells appear in e12, somatostatin producing cells appear at e17. (See Myrsén-Axcrona et al., *supra*). It is thought that pancreatic islet cells differentiate in response to endocrine signals from a common precursor cell in the pancreatic ducts. Sometime between the end of the fetal stage (e21) and neonatal stages (post-birth) the fetal β cells acquire the ability to secrete insulin in response to glucose. The insulin response at this age is monophasic and is not blocked by Ca^{2-} antagonists. A clear biphasic pattern of insulin secretion in response to glucose is detected only 3 days after birth. (Mendonca, A.C. et al. (1998) *Brazilian J. Med. Biol. Res.* 31(6):841-46). The mechanism by which this "gain of function" or "gain of glucose responsivity" is achieved is not known, nor have the factors that regulate the maturation and gain of function been identified or characterized. In addition, the physiological changes associated with gain of glucose responsivity in pancreatic β cells are not known. Such mechanisms, factors, and changes could be useful for developing therapies for insulin-related diseases such as diabetes.

Summary of the Invention

The invention relates to the discovery that secretin therapeutics modulate the growth state of insulin positive progenitor cells. Secretin therapeutics have been found to induce insulin-positive progenitor cells in pancreatic duct cultures. It has been found that secretin therapeutics also induce differentiation in ductal epithelial cells. For instance, it has been observed that insufficient levels of insulin allow full differentiation of the epithelial ductal cells to occur until the insulin levels have been restored. It has also been observed that increased levels of insulin block this inductive activity.

In one embodiment, the invention comprises a method for inducing insulin-positive progenitor cells, comprising administering to cultures of ductal epithelial cells

secretin therapeutics, such as an analog or derivative of secretin therapeutic which emulates the activity of the secretin therapeutic, such as a secretin agonist. Administration of the secretin therapeutic may cause proliferation of the progenitor cells.

In yet another embodiment, the invention features a pharmaceutical composition including as the cellular component, a substantially pure population of viable pancreatic progenitor cells and a secretin therapeutic, which progenitor cells are capable of proliferation in a culture medium.

In general, the preferred progenitor cells will be of mammalian origin, e.g., cells isolated from a primate such as a human, from a miniature swine, or from a transgenic mammal, or are the cell culture progeny of such cells. In one embodiment, pancreatic ductal tissue is isolated from a patient and subjected to the present method to provide a resulting culture of pancreatic progenitor cells (or differentiated cells derived therefrom). Gene replacement or other gene therapy is carried out *ex vivo*, and the isolated cells are transplanted back into the initial donor patient or into a second host patient.

In another aspect, the invention features a cellular composition comprising, as a cellular population, at least 75% (though more preferably at least 80, 90, or 95%) progenitor cells and capable of self-regeneration and/or renewal in a culture medium.

In yet another aspect, the invention features a cellular composition consisting essentially of, as the cellular population, viable pancreatic progenitor cells capable of self-regeneration and/or self-renewal in a culture medium and of differentiation to pancreatic lineages in the presence of a secretin therapeutic. For instance, in certain embodiments the progenitor cells are isolated from pancreatic intralobular duct explants, e.g., isolated by biopsy, or are the cell culture progeny of such cells.

One aspect of the invention features a method for treating isolated pancreatic progenitor cells from a sample of pancreatic duct. In general, the method provides for a culture system that allows reproducible expansion of pancreatic ductal epithelium while maintaining "stemmedness" and the ability to differentiate into endocrine and exocrine cells. As illustrated below, in a preferred embodiment, pancreatic ductal epithelium is obtained, e.g., by explant or enzymatic digestion, and cultured to confluence. The confluent cell population is contacted with an agent, e.g., a secretin therapeutic, which induces insulin positive progenitor cells in the cultured population. Subsequently, progenitor cells from the explant that proliferate in response to the secretin therapeutics

are isolated, such as by direct mechanical separation of newly emerging buds from the rest of the explant or by dissolution of all or a portion of the explant and subsequent isolation of the progenitor cell population.

In another aspect, this invention comprises a method for inducing differentiation of ductal epithelial cells. It has been observed that insufficient levels of insulin allow full differentiation to take place until insulin levels are restored. However, adequate levels of insulin result in the abrogation of this activity. Accordingly, one aspect of this invention provides a method of screening for compounds that inhibit the insulin inhibition or abrogation of secretin therapeutic activity.

In yet another preferred embodiment, the pancreatic progenitor cells are induced to differentiate into pancreatic islet cells, e.g., β islet cells, α islet cells, δ islet cells, or ϕ islet cells, subsequent to being introduced into the subject. Preferably, the pancreatic progenitors cells are induced to differentiate into pancreatic isles, e.g., β islet cells, α islet cells, δ islet cells, or ϕ islet cells, in culture prior to introduction into the subject.

In another aspect, the invention comprises a method for altering the differentiated state of pancreatic islet cells, comprising administering to the pancreatic islets, or isolated β cells, a secretin therapeutic, an analog or derivative of a secretin therapeutic which emulates the activity of a secretin therapeutic, or a secretin agonist (collectively referred to herein "secretin therapeutics"). In one embodiment, administration of a secretin therapeutic causes the islets or cells to be insulin-positive. The islets or cells, such as progenitor cells are thereby stimulated to produce insulin when exposed to secretin. In another aspect, the invention comprises methods for inducing islets to express markers indicative of mature islets, or for β cells to express markers indicative of mature β cells by contacting the islets or β cells. In a preferred embodiment, the islets or cells are human islets or cells.

The invention further provides methods for preparing functional (i.e., insulin producing) pancreatic islets or β cells, comprising administering to secretin responsive cells an effective amount of a composition comprising a secretin therapeutic.

In another aspect, the invention provides a method for modifying glucose metabolism in an animal, comprising administering to the animal a pharmaceutically effective amount of a composition comprising a secretin therapeutics and a pharmaceutically acceptable carrier. The secretin therapeutic may enhance

responsiveness of pancreatic cells, or promote insulin expression or production. In a preferred embodiment, the invention provides a method for treating a disease associated with altered glucose metabolism, comprising administering to an animal a pharmaceutically effective amount of a composition comprising a secretin therapeutic and a pharmaceutically acceptable carrier, in an amount sufficient to increase the level of insulin. In yet another embodiment, a secretin therapeutic boosts the insulin content of weakly insulin-positive cells.

In still another aspect, the invention provides a method for treating a disease associated with altered glucose metabolism, comprising administering to an animal a pharmaceutically effective amount of a composition comprising pancreatic cells responsive to treatment with a secretin therapeutic according to the invention. In one embodiment, the insulin-positive progenitor cells obtained by treating pancreatic cells with secretin therapeutics are administered to an animal in a composition containing a pharmaceutically acceptable carrier in an amount sufficient to increase the responsiveness of pancreatic cells. In another embodiment, the composition further comprises additional agents, such as a secretin therapeutic. The cell composition may be conjointly administered simultaneously, sequentially, or separately with a secretin therapeutic. The method may be used for treating a disease that is associated with a condition such as insulin resistance, glucose intolerance or glucose non-responsiveness, hyperglycemia, hyperinsulinemia, obesity, hyperlipidemia, or hyperlipoproteinemia in an animal. In a preferred embodiment, the method is used to treat Type II diabetes mellitus.

Secretin agonists which can be used as secretin therapeutics include any compound having the effect of inducing the activity of secretin therapeutics. These therapeutics include but are not limited to dexamethasone, dibutyryl-cAMP, Na-butyrate, forskolin, or cholera toxin.

In yet another embodiment, the invention provides a method for inducing proliferation of insulin positive progenitor cells in cell transplants, comprising administering a secretin therapeutic ex vivo to ductal epithelial cells. A salient feature of the subject method concerns the use of defined explants as sources from which discrete progenitor cells populations may be amplified.

Accordingly, small samples of pancreatic tissue from a donor can be obtained without sacrificing or seriously injuring the donor. The progenitor cells of the present invention can be amplified, and subsequently isolated from the epithelial explant, based on a proliferative response upon, for example, addition of defined growth factors or biological extracts to the culture.

In another embodiment, the invention provides a method for maintaining normal pancreatic islet function (i.e., insulin production) in islet or cell transplants, comprising administering to *ex vivo* pancreatic islets or cells secretin therapeutics. In this way, donor pancreatic islets or cells that are to be transplanted into a host animal can be maintained as functional with respect to their ability to respond to secretin by producing insulin. Alternatively, the pancreatic islet cells may be autologous failed β cells of the host which are treated with secretin therapeutics to enrich for secretin-responsive cells or to increase their insulin content prior to reimplantion into the animal.

In another aspect, the invention features, a method for screening a compound for ability to modulate one of growth, proliferation, and/or differentiation of progenitor cells obtained by the subject method, including: (i) establishing an isolated population of pancreatic progenitor cells; (ii) adding secretin therapeutics to the population of cells; (iii) contacting the population of cells with a test compound; and (iv) detecting one of growth, proliferation, and/or differentiation of the progenitor cells in the population, wherein a statistically significant change in the extent of one of growth, proliferation, and/or differentiation in the presence of the test compound relative to the extent of one of growth, proliferation, and/or differentiation in the absence of the test compound indicates the ability of the test compound to modulate one of the growth, proliferation, and/or differentiation.

Thus, in one embodiment, the invention comprises a method for modulating the growth state of pancreatic cells comprising contacting the cells with a secretin therapeutic or prodrug form thereof. In another embodiment, the invention comprises a method for promoting proliferation of pancreatic cells comprising contacting the cells with a secretin therapeutic or prodrug form thereof. In such methods, the secretin therapeutic may be provided as part of a composition also comprising a pharmaceutically acceptable excipient. In such methods, the method may be performed *in vitro*. The pancreatic cells may comprise pancreatic tissue, epithelial cells, ductal

epithelial cells, or any other cells, particularly those capable of expressing insulin, e.g., when contacted with secretin or a secretin therapeutic. In certain embodiments, the secretin therapeutic comprises a polypeptide sequence at least 60% identical to SEQ ID No. 2 or an active fragment thereof; a secretin peptidomimetic, such as a derivative of a polypeptide at least 60% identical to SEQ ID No. 2 wherein one or more amide bonds is replaced with a protease-resistant bond, whereby the peptidomimetic has a serum half-life longer than the peptide represented in SEQ ID No. 2; a non-peptidyl secretin agonist, such as a small organic molecule; or any other compound capable of mimicking the effect of secretin on a pancreatic cell.

In another embodiment. the invention comprises a method for modifying glucose metabolism in an animal by administering to the animal a pharmaceutically effective amount of a secretin therapeutic. In yet another embodiment, the invention comprises a method for treating a disease associated with altered glucose metabolism by administering to an animal a pharmaceutically effective amount of a secretin therapeutic. In still another embodiment, the invention comprises method for treating a disease associated with altered glucose metabolism by administering to an animal a secretin therapeutic and a pharmaceutically effective amount of a composition comprising secretin-responsive islets or cells, wherein the therapeutic and the islets or cells are administered either simultaneously or sequentially. In certain embodiments, the disease may be associated with a condition selected from the group consisting of insulin resistance, glucose intolerance or glucose non-responsiveness, hyperglycemia, hyperinsulinemia, obesity, hyperlipidemia and hyperlipoproteinemia in a subject, such as Type II diabetes mellitus (NIDD). In yet another embodiment, the invention comprises a method for generating a functional pancreatic β cell by contacting a pancreatic islet or cell with a secretin therapeutic. In any of these methods, the pancreatic islet or cell may be contacted with a secretin therapeutic *in vitro*. The secretin therapeutic may be provided as part of a composition also including a pharmaceutically acceptable excipient. In certain embodiments, the secretin therapeutic promotes insulin production in a pancreatic islet or cell, antagonizes insulin inhibition of secretin response in secretin-responsive cells, includes an agent capable of inhibiting the degradation of secretin or a secretin agonist, binds to a secretin-responsive receptor, is a vector comprising a nucleic acid encoding a polypeptide at least 60% identical to SEQ ID No. 2

or a biologically active fragment thereof, is a small organic molecule, or is otherwise designed to augment, recapitulate, or mimic the biological effect of secretin. Any of the above methods may further include administering an agent capable of inhibiting the degradation of secretin or a secretin agonist, e.g., administered simultaneously with secretin or a secretin agonist. Any of the above methods may also include administering to the animal insulin, a dipeptidylpeptidase inhibitor, or GLP-1.

In yet another aspect, the invention comprises a method for isolating secretin-responsive cells by providing a sample of cells, identifying in the sample cells responsive to secretin, and isolating the identified cells from the sample of cells. The sample of cells may include pancreatic cells, pancreatic ductal epithelial cells, or other cells. Cells responsive to secretin may be identified by contacting the sample of cells with secretin or a secretin agonist and identifying cells characterized by an increased rate of proliferation in the presence of the secretin or the secretin agonist; by contacting the sample of cells with secretin or a secretin agonist and identifying cells characterized by an increased concentration of insulin in the presence of the secretin or the secretin agonist; by contacting the sample of cells with an antibody immunoreactive with a secretin-responsive receptor and identifying cells bound by the antibody; by contacting the sample of cells with secretin or a secretin agonist and identifying cells characterized by an altered growth state in the presence of the secretin or the secretin agonist; by contacting the sample of cells with a detectably labelled derivative of secretin and identifying cells bound by the detectably labelled derivative of secretin; or by any other suitable means. Isolating the identified cells may include separating the cells using an automatic cell sorter. The invention may also comprise a substantially pure sample of cells isolated by the method of claim 30 or a substantially pure sample of pancreatic progenitor cells which respond to secretin by either increasing insulin production or by proliferating at an increased rate. The invention further includes a composition comprising any such cells and a pharmaceutically acceptable carrier or a biocompatible polymer.

In yet another embodiment, the invention provides a method for identifying a secretin therapeutic by providing a sample of cells, treating the sample of cells with a test agent, and

comparing the sample of cells to an control sample of cells in the absence of the test

agent, wherein an increase in the insulin concentration, the insulin expression, the rate of proliferation, or the rate of differentiation of the treated cells, relative to the untreated cells, is indicative of secretin therapeutic activity. The sample of cells may include pancreatic cells, insulin-positive progenitor cells, or any other suitable cells. The invention also includes a secretin therapeutic identified by such a method.

In yet another embodiment, the invention includes a method for identifying a secretin antagonist by providing a sample of cells, treating the sample of cells with a test agent, treating the sample of cells with secretin or a secretin agonist, and comparing the sample of cells to a control sample of cells treated with secretin in the absence of the test agent, wherein an increase in the insulin concentration, the insulin expression, the rate of proliferation, or the rate of differentiation of the control cells, relative to the treated cells, is indicative of secretin antagonist activity. The sample of cells may include pancreatic cells, insulin-positive progenitor cells, or any other suitable cells. The invention also includes a secretin antagonist identified by such a method.

In yet another embodiment, the invention comprises a method for identifying an agent which antagonizes insulin inhibition of secretin response by providing a sample of cells, treating the sample of cells with insulin, treating the sample of cells with secretin or a secretin agonist, treating the sample of cells with a test agent, and comparing the sample of cells with a control sample of cells, wherein an increase in the insulin concentration, the insulin expression, the rate of proliferation, or the rate of differentiation of the treated cells, relative to the control sample of cells, is indicative of an agent which antagonizes insulin inhibition of secretin response. The control sample of cells may be treated with secretin and insulin in the absence of the test agent, may be maintained in the absence of secretin, or may be cultured in the presence of any compounds or reagents that provide a suitable control for the assay. The sample of cells may include pancreatic cells, insulin-positive progenitor cells, or any other suitable cells. The invention also includes a compound identified by such a method.

In the various assays and methods of the present invention, a difference between the test and control cells of a measured characteristic which is statistically significant, i.e., differs by at least a factor of two, at least a factor of five, or at least a factor of ten, may indicate an activity of the test compound.

In yet another aspect, the invention provides a composition comprising any compound identified in the above assays and pharmaceutically acceptable excipient. In certain embodiments, such a composition may further include a substantially pure sample of cells which respond to secretin by either increasing insulin production or by proliferating at an increased rate. The cells may include pancreatic cells, insulin-positive progenitor cells, or any other suitable cells.

Brief Description of the Figures

Figure 1 presents a graph indicating populations of cells and of insulin-responsive cells in particular.

Figure 2 shows results indicating low contamination of insulin-responsive cells in a purified populations.

Figure 3 indicates populations of floating progenitor cells relative to total cells in a culture.

Figure 4 presents results indicating populations of seeded cells and floating progenitor cells after separation.

Figure 5 indicates results from an assay testing various compounds at various concentrations for induction of differentiation.

Figure 6 demonstrates the induction of insulin production by DCE.

Figure 7. The average number of non-adherent cells (NACs) per well increases with cAMP treatment. Duct monolayers were treated at day five with the following concentrations of factors: Na-butyrate 25 μM, 12.5 μM (1,2); Forskolin 20, 10, 5, 1 μM (1-4); Control (5% fetal calf serum (FCS)); DCE dexamethasone 100 μM, cholera toxin 1 μM, EGF 10 ng/ml; CPT 50, 25, 25, 12.5 μM (1-4), for 48 hours. The nonadherent cells were collected, washed, counted, and processed for insulin content by ELISA

analysis. Treatment with DCE increased the number of adherent cells by 5.5 fold (18.3 x 103 vs. 3.3 x 103 cells per well). The effect of DCE could be mimicked by the singular addition of cAMP modulating compounds such as forskolin and the cAMP analog 8-(4-chlorophenylthio)adenosine-3':5'-cyclic monophosphate (CPT). Sodium butyrate, which has been shown to be a stimulator of differentiation in a number of systems could also mimic the DCE effect.

Figure 8. cAMP analogs also increase insulin content in NACs. After harvest and counting, NACs were processed for insulin content analysis by ELISA. Data is expressed as mean of triplicates plus standard deviation. Insulin content is measured in total pg per NACs harvested per well. Note that while DCE induces five times more NACs than control, the overall insulin content is similar. In contrast, the cAMP modulating agents not only increase overall cell number, but also increase the overall insulin content as well, thus increasing the amount of insulin on a per cell basis.

Figure 9. Comparison of insulin content in secretin-induced NACs. Duct monolayers were grown for five days in 5% FCS and then cultured an additional 2 days after addition of the indicated factors. Secretin was tested at 100, 75, and 50 nM, VIP also at 100, 75, and 50 nM, and dibutyryl cAMP, a cAMP agonist, at 50, 25, 12.5, and 1 nM. Secretin at 50 nM gave the highest rise in NAC insulin content. The effect of VIP was less marked. Higher concentrations of dibutyryl cAMP could mimic the effect of secretin.

Figure 10. Average number of NACs per well per condition. There is no significant difference in the number of NACs observed in each condition despite the wide differences in insulin content (Figure 9). Concentrations of factors are as indicated in Figure 9. Therefore, the differences in insulin content in Figure 9 reflect true per-cell content differences with secretin at 50 nM being the most potent in this experiment.

Detailed Description of the Invention

(i) Overview of the Invention

During the early stages of embryogenesis, cells are totipotent and are capable of multidirectional differentiation. As development proceeds, the totipotent cells become determined and committed to differentiate into a given specialized cell type. Final differentiation is associated with the acquisition of specialized cell functions. Thus, the differentiated somatic cells maintain their specialized features throughout the life span of the organism, probably through sustained interactions between the genome and its microenvironment and cell-cell interactions (DiBerardino et al., 1984, *Science* 224:946-952; Wetts and Fraser, 1988, *Science* 239:1142-1144; Fisher, 1984, *PNAS* 81:4414-4418).

Because of the tremendous potential of progenitor cells to differentiate into distinct lineages, there has always existed a need for a continuous source of these isolated pluripotent progenitor cells. The pluripotent progenitor cells could be extremely useful in the treatment of different disorders that are characterized by insufficient or abnormal functioning of the fully differentiated cells in a given organ, such as in the human pancreas or liver.

The ability to isolate distinct populations of progenitor cells has been an important problem in modern biology. It can be easily envisioned that such isolated pluripotent progenitor cells could be useful for treatment of various disorders associated with loss or abnormal functioning of fully differentiated cells in a given organ. For example, the ability to introduce isolated progenitor cells capable of subsequent differentiation, either in culture or when introduced into a subject, into functional β islet cells, would have important implications for the treatment of insulin-dependent diabetes.

Accordingly, certain aspects of the present invention relate to isolated populations of progenitor cells capable of subsequent differentiation to distinct pancreatic lineages, methods for isolating such cells and therapeutic uses for such cells.

(ii) Definitions

For convenience, certain terms employed in the specification, examples, and appended claims are collected here.

The term "agonist", as used herein, is meant to refer to an agent that upregulates (e.g. mimics potentiates or enhances) a secretin therapeutic bioactivity. A secretin

agonist can be a wild-type secretin protein or derivative thereof having at least one bioactivity of a wild-type secretin protein. A secretin agonist can also be a compound that upregulates expression of a secretin gene or which increases at least one bioactivity of a secretin protein. Secretin agonists
therefore may include those agents that upregulate the production and/or secretion of insulin. An agonist can also be a compound that upregulates expression of a secretin or insulin gene or which increases the amount of secretin or insulin protein present, e.g., by increasing protein synthesis or decreasing protein turnover. Such agonists may include but are not limited to compounds such as dexamethasone, dibutyryl-cAMP, Na-butyrate, forskolin, or cholera toxin.

The term "antagonist" as used herein is meant to refer to an agent that downregulates (e.g., suppresses or inhibits) at least one secretin bioactivity. A secretin antagonist can be a compound which inhibits or decreases the interaction between a secretin protein and another molecule, e.g., a secretin receptor. Alternatively, a preferred antagonist is a compound which inhibits or decreases binding of a secretin or secretin receptor transcription factor to the upstream region of a secretin therapeutic therapeutics or secretin receptor gene, or of an insulin gene transcription factor to the upstream region of an insulin gene, thereby blocking the synthesis of the insulin protein. An antagonist can also be a compound that downregulates expression of a secretin or insulin gene or which induces the amount of secretin or insulin protein present, e.g., by decreasing protein synthesis or increasing protein turnover. The secretin antagonist can be a dominant negative form of a secretin polypeptide. The secretin antagonist can also be a nucleic acid encoding a dominant negative form of a secretin polypeptide, an antisense nucleic acid to a secretin therapeutic, or a ribozyme capable of interacting specifically with a secretin therapeutic RNA. Yet other secretin therapeutic antagonists are molecules which bind to a secretin polypeptide or its receptor and inhibit its action. Such molecules include peptides, antibodies and small molecules.

"Biological activity" or "bioactivity" or "activity" or "biological function", which are used interchangeably, for the purposes herein means an effector or antigenic function that is directly or indirectly performed by a secretin polypeptide (whether in its native or denatured conformation), or by any subsequence thereof. Biological activities include modulating the growth state of an insulin positive progenitor cell.

The term "antibody" as used herein is intended to include whole antibodies, e.g., of any isotype (IgG, IgA, IgM, IgE, etc), and includes fragments thereof which are also specifically reactive with a vertebrate, e.g., mammalian, protein. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. Thus, the term includes segments of proteolytically-cleaved or recombinantly-prepared portions of an antibody molecule that are capable of selectively reacting with a certain protein. Nonlimiting examples of such proteolytic and/or recombinant fragments include Fab, F(ab')2, Fab', Fv, and single chain antibodies (scFv) containing a V[L] and/or V[H] domain joined by a peptide linker. The scFv's may be covalently or non-covalently linked to form antibodies having two or more binding sites. The subject invention includes polyclonal, monoclonal, or other purified preparations of antibodies and recombinant antibodies.

"Antisense" nucleic acids refer to nucleic acids that specifically hybridize (e.g., bind) with cellular mRNA and/or genomic DNA under cellular conditions so as to inhibit expression (e.g., by inhibiting transcription and/or translation). The binding may be by conventional base pair complementarity or, for example, in the case of binding to DNA duplexes, through specific interactions in the major groove of the double helix.

The term "culture medium" is recognized in the art, and refers generally to any substance or preparation used for the cultivation of living cells. Accordingly, a "tissue culture" refers to the maintenance or growth of tissue, e.g., explants of organ primordia or of an adult organ *in vitro* so as to preserve its architecture and function. A "cell culture" refers to a growth of cells *in vitro*; although the cells proliferate, they do not organize into tissue *per se*.

Tissue and cell culture preparations of micro-organ explants and amplified progenitor cell populations can take on a variety of formats. For instance, a "suspension culture" refers to a culture in which cells multiply while suspended in a suitable medium. Likewise, a "continuous flow culture" refers to the cultivation of cells or ductal explants in a continuous flow of fresh medium to maintain cell growth, e.g., viablity. The term "conditioned media" refers to the supernatant, e.g. free of the cultured cells/tissue, resulting after a period of time in contact with the cultured cells such that the media has been altered to include certain paracrine and/or autocrine factors produced by the cells and secreted into the culture.

The term "explant" refers to a portion of an organ taken from the body and grown in an artificial medium.

"Cells," "host cells", or "recombinant host cells" are terms used interchangeably herein. It is understood that such terms refer not only to the particular subject cell, but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A "chimeric polypeptide" or "fusion polypeptide" is a fusion of a first amino acid sequence encoding one of the subject polypeptides with a second amino acid sequence defining a domain (e.g., polypeptide portion) foreign to and not substantially homologous with any domain of the subject polypeptide. A chimeric polypeptide may present a foreign domain which is found (albeit in a different polypeptide) in an organism which also expresses the first polypeptide, or it may be an "interspecies," "intergenic," etc., fusion of polypeptide structures expressed by different kinds of organisms. In general, a fusion polypeptide can be represented by the general formula (X)n-(Y)m-(Z)n, wherein Y represents a portion of the subject polypeptide, and X and Z are each independently absent or represent amino acid sequences which are not related to the native sequence found in an organism, or which are not found as a polypeptide chain contiguous with the subject sequence, where m is an integer greater than or equal to one, and each occurrence of n is, independently, 0 or an integer greater than or equal to 1 (n and m are preferably no greater than 5 or 10).

A "coding sequence" refers to a nucleic acid sequence or portion thereof which encodes the amino acid sequence of a protein or polypeptide, e.g., which does not include intronic sequences.

A "delivery complex" shall mean a targeting means (e.g., a molecule that results in higher affinity binding of a nucleic acid, protein, polypeptide or peptide to a target cell surface and/or increased cellular or nuclear uptake by a target cell). Examples of targeting means include: sterols (e.g., cholesterol), lipids (e.g., a cationic lipid, virosome or liposome), viruses (e.g., adenovirus, adeno-associated virus, and retrovirus), or target cell-specific binding agents (e.g., ligands recognized by target cell specific receptors).

Preferred complexes are sufficiently stable in vivo to prevent significant uncoupling prior to internalization by the target cell. However, the complex is cleavable under appropriate conditions within the cell so that the nucleic acid, protein, polypeptide or peptide is released in a functional form.

"Homology" or "identity" or "similarity" refers to sequence similarity between two peptides or between two nucleic acid molecules, with identity being a more strict comparison. Homology and identity can each be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are identical at that position. A degree of homology or similarity or identity between nucleic acid sequences is a function of the number of identical nucleotides at positions shared by the nucleic acid sequences. A degree of identity of amino acid sequences is a function of the number of identical amino acids at positions shared by the amino acid sequences. A degree of homology or similarity of amino acid sequences is a function of the number of structurally related amino acids at positions shared by the amino acid sequences. When an equivalent position in the compared sequences is occupied by the same base or amino acid, then the molecules are identical at that position; when the equivalent site occupied by the same or a similar amino acid residue (e.g., similar in steric and/or electronic nature), then the molecules can be referred to as homologous (similar) at that position. Expression as a percentage of homology, similarity, or identity refers to a function of the number of identical or similar amino acids at positions shared by the compared sequences. An "unrelated" or "non-homologous" sequence shares less than 40% identity, though preferably less than 25% identity, with one of the sequences of the present invention.

The term "insulin-positive progenitor cell" as used herein includes cells that are secretin-responsive precursors of pancreatic cells, such as exocrine or endocrine cells, in particular, α-islet cells, β-islet cells, ϕ-islet cells, and δ-islet cells.

As is well known, genes or a particular polypeptide may exist in single or multiple copies within the genome of an individual. Such duplicate genes may be identical or may have certain modifications, including nucleotide substitutions, additions or deletions, which all still code for polypeptides having substantially the same activity.

The term "DNA sequence encoding a polypeptide" may thus refer to one or more genes within a particular individual. Moreover, certain differences in nucleotide sequences may exist between individual organisms, which are called alleles. Such allelic differences may or may not result in differences in amino acid sequence of the encoded polypeptide yet still encode a polypeptide with the same biological activity.

The term "percent identical" refers to sequence identity between two amino acid sequences or between two nucleotide sequences. Identity can each be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When an equivalent position in the compared sequences is occupied by the same base or amino acid, then the molecules are identical at that position; when the position is occupied by the same or a similar amino acid residue (e.g., similar in steric and/or electronic nature), then the molecules can be referred to as homologous (similar) at that position. Expression as a percentage of homology, similarity, or identity refers to a function of the number of identical or similar (including identical) amino acids at positions shared by the compared sequences. Various alignment algorithms and/or programs may be used, including FASTA, BLAST, or ENTREZ. FASTA and BLAST are available as a part of the GCG sequence analysis package (University of Wisconsin, Madison, Wis.), and can be used, e.g., with default settings. ENTREZ is available through the National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, Md. In one embodiment, the percent identity of two sequences can be determined by the GCG program with a gap weight of 1, e.g., each amino acid gap is weighted as if it were a single amino acid or nucleotide mismatch between the two sequences. Preferred nucleic acids have a sequence at least 70%, and more preferably 80% homologous and more preferably 90% and even more preferably at least 95% homologous to an nucleic acid sequence of a sequence shown in one of SEQ ID NOS: 1 or 3. In certain embodiments, preferred nucleic acids have a sequence at least 70%, and more preferably 80% identical and more preferably 90% and even more preferably at least 95% identical to an nucleic acid sequence of a sequence shown in one of SEQ ID NOS: 1-13. Nucleic acids at least 90%, more preferably 95%, and most preferably at least about 98-99% identical with a nucleic sequence represented in one of SEQ ID NOS: 1 or 3 are of course also within the scope of the invention. In

preferred embodiments. the nucleic acid is mammalian.

As used herein. the term "nucleic acid" refers to polynucleotides such as deoxyribonucleic acid (DNA), and. where appropriate, ribonucleic acid (RNA). The term should also be understood to include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs, and. as applicable to the embodiment being described. single (sense or antisense) and double-stranded polynucleotides. ESTs. chromosomes. cDNAs. mRNAs. and rRNAs are representative examples of molecules that may be referred to as nucleic acids.

The term "nucleotide sequence complementary to the nucleotide sequence of SEQ ID NO. x" refers to the nucleotide sequence of the complementary strand of a nucleic acid strand having SEQ ID NO. x. The term "complementary strand" is used herein interchangeably with the term "complement". The complement of a nucleic acid strand can be the complement of a coding strand or the complement of a non-coding strand.

The term "secretin therapeutic" as used herein includes agents which emulate the activity of wild-type secretin or a derivative thereof having at least one bioactivity of the wild-type secretin, an analog, or derivative of secretin therapeutics. Included are secretin agonists and compounds such as dexamethasone, dibutyryl-cAMP, Na-butyrate, forskolin, and cholera toxin, which mimic certain aspects of secretin bioactivity, as well as prodrug forms of active secretin therapeutics, e.g., compounds which, under physiological conditions, e.g., in the stomach, in the bloodstream, etc., undergoes a rearrangement, transformation, or reaction that results in a compound having increased secretin therapeutic or secretin agonist activity. A secretin therapeutic may further comprise a vector for transfecting a cell with a nucleic acid sequence, e.g., SEQ ID No. 1 or a fragment or variant thereof, encoding secretin or a bioactive fragment thereof, e.g., SEQ ID No. 2 or a fragment thereof. In preferred embodiments, a secretin therapeutic may be any compound, preferably secretin, an active fragment thereof, a peptidomimetic thereof, or a small organic molecule, which induces differentiation or proliferation of pancreatic ductal epithelial cells and/or increases the cellular concentration or expression of insulin in pancreatic ductal epithelial cells. In certain preferred embodiments, a secretin therapeutic is capable of binding and activating a receptor bound and activated

by secretin, such as secretin receptor (e.g., SEQ ID No. 4, encoded by SEQ ID No. 3), and/or shares an activity with secretin that is inhibited by insulin.

The term "tissue" refers to a group or layer of similarly specialized cells which together perform certain special functions.

The term "organ" refers to two or more adjacent layers of tissue, which layers of tissue maintain some form of cell-cell and/or cell-matrix interaction to form a microarchitecture.

The term "lineage committed cell" refers to a progenitor cell that is no longer pluripotent but has been induced to differentiate into a specific cell type, e.g., a pancreatic cell.

The term "progenitor cell" refers to an undifferentiated cell which is capable of proliferation and giving rise to more progenitor cells having the ability to generate a large number of mother cells that can in turn give rise to differentiated, or differentiable daughter cells. As used herein, the term "progenitor cell" is also intended to encompass a cell which is sometimes referred to in the art as a "stem cell". In a preferred embodiment, the term "progenitor cell" refers to a generalized mother cell whose descendants (progeny) specialize, often in different directions, by differentiation, e.g., by acquiring completely individual characters, as occurs in progressive diversification of embryonic cells and tissues. "Progenitor cells" refers to progenitor cells arising in tissue of a pancreatic intralobular duct and giving rise to such differentiated progeny as, for example, β cell lineages.

The term "pancreatic duct" includes the accessory pancreatic duct, dorsal pancreatic duct, main pancreatic duct and ventral pancreatic duct.

As used herein the term "substantially pure", with respect to progenitor cells, refers to a population of progenitor cells that is at least about 75%, preferably at least about 85%, more preferably at least about 90%, and most preferably at least about 95% pure, with respect to progenitor cells making up a total cell population. Recast, the term "substantially pure" refers to a population of progenitor cells of the present invention that contains fewer than about 20%, more preferably fewer than about 10%, most preferably fewer than about 5%, of lineage-committed cells in the original unamplified and isolated population prior to subsequent culturing and amplification.

A "peptidomimetic", as the term is used herein, refers to an analog of a polypeptide which is resistant to biological degradation, such as degradation by

peptidase or protease enzymes. Peptidomimetics can be generate by synthesizing retro-inverso or retro-enantio analogs of a polypeptide, by replacing amide carbonyls with methylene groups (substituted or unsubstituted), by replacing amide groups along the backbone with olefins, phosphonates, phosphonamids, or heterocyclic groups, such as oxazole rings, or by any other substitution that results in a molecule which displays a similar array of functional groups in a similar disposition, for example by bearing appendages corresponding to the side chains of the polypeptide, but which has a half-life under physiological conditions greater than that of the corresponding polypeptide, e.g., by resisting enzymatic degradation. Retro-enantio analogs can be synthesized using D-enantiomers of commercially available D-amino acids or other amino acid analogs and standard solid- or solution-phase peptide-synthesis techniques. The side-chains of the resulting peptide are coincident in space with the sidechains of the L-amino acid peptide, though the backbone amide is reversed, rendering that bond resistant to cleavage.

As used herein, the term "cellular composition" refers to a preparation of cells, which preparation may include, in addition to the cells, non-cellular components such as cell culture media, e.g. proteins, amino acids, nucleic acids, nucleotides, co-enzyme, anti-oxidants, metals and the like. Furthermore, the cellular composition can have components which do not affect the growth or viability of the cellular component, but which are used to provide the cells in a particular format, e.g., as polymeric matrix for encapsulation or a pharmaceutical preparation.

As used herein the term "animal" refers to mammals, preferably mammals such as humans. Likewise, a "patient" or "subject" to be treated by the method of the invention can mean either a human or non-human animal.

As used herein, the term "promoter" means a DNA sequence that regulates expression of a selected DNA sequence operably linked to the promoter, and which effects expression of the selected DNA sequence in cells. The term encompasses "tissue specific" promoters, i.e., promoters which effect expression of the selected DNA sequence only in specific cells (e.g., cells of a specific tissue). The term also covers so-called "leaky" promoters, which regulate expression of a selected DNA primarily in one tissue, but cause expression in other tissues as well. The term also encompasses non-tissue specific promoters and promoters that constitutively express or that are inducible (i.e., expression levels can be controlled).

The terms "protein", "polypeptide", and "peptide" are used interchangeably herein when referring to a gene product.

The term "recombinant protein" refers to a polypeptide of the present invention which is produced by recombinant DNA techniques, wherein generally, DNA encoding a polypeptide is inserted into a suitable expression vector which is in turn used to transform a host cell to produce the heterologous protein. Moreover, the phrase "derived from", with respect to a recombinant gene, is meant to include within the meaning of "recombinant protein" those proteins having an amino acid sequence of a native polypeptide, or an amino acid sequence similar thereto which is generated by mutations including substitutions and deletions (including truncation) of a naturally occurring form of the polypeptide.

As used herein, the term "specifically hybridizes" or "specifically detects" refers to the ability of a nucleic acid molecule of the invention to hybridize to at least a portion of, for example, approximately 6, 12, 15, 20, 30, 50, 100, 150, 200, 300, 350, 400, 500, 750, or 1000 contiguous nucleotides of a nucleic acid designated in any one of SEQ ID Nos: 1 or 3, or a sequence complementary thereto, or naturally occurring mutants thereof, such that it has less than 15%, preferably less than 10%, and more preferably less than 5% background hybridization to a cellular nucleic acid (e.g., mRNA or genomic DNA) encoding a different protein. In preferred embodiments, the oligonucleotide probe detects only a specific nucleic acid, e.g., it does not substantially hybridize to similar or related nucleic acids, or complements thereof.

"Transcriptional regulatory sequence" is a generic term used throughout the specification to refer to DNA sequences, such as initiation signals, enhancers, and promoters, which induce or control transcription of protein coding sequences with which they are operably linked. In preferred embodiments, transcription of one of the genes is under the control of a promoter sequence (or other transcriptional regulatory sequence) which controls the expression of the recombinant gene in a cell-type in which expression is intended. It will also be understood that the recombinant gene can be under the control of transcriptional regulatory sequences which are the same or which are different from those sequences which control transcription of the naturally-occurring forms of the polypeptide.

As used herein, the term "transfection" means the introduction of a nucleic acid, e.g., via an expression vector, into a recipient cell by nucleic acid-mediated gene transfer.

"Transformation", as used herein, refers to a process in which a cell's genotype is changed as a result of the cellular uptake of exogenous DNA or RNA, and, for example, the transformed cell expresses a recombinant form of a polypeptide or, in the case of anti-sense expression from the transferred gene, the expression of the target gene is disrupted.

As used herein, the term "transgene" means a nucleic acid sequence (or an antisense transcript thereto) which has been introduced into a cell. A transgene could be partly or entirely heterologous, i.e., foreign, to the transgenic animal or cell into which it is introduced, or, is homologous to an endogenous gene of the transgenic animal or cell into which it is introduced, but which is designed to be inserted, or is inserted, into the animal's genome in such a way as to alter the genome of the cell into which it is inserted (e.g., it is inserted at a location which differs from that of the natural gene or its insertion results in a knockout). A transgene can also be present in a cell in the form of an episome. A transgene can include one or more transcriptional regulatory sequences and any other nucleic acid, such as introns, that may be necessary for optimal expression of a selected nucleic acid.

The term "treating" as used herein is intended to encompass curing as well as ameliorating at least one symptom of the condition or disease.

The term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of preferred vector is an episome, i.e., a nucleic acid capable of extra-chromosomal replication. Preferred vectors are those capable of autonomous replication and/or expression of nucleic acids to which they are linked. Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of "plasmids" which refer generally to circular double stranded DNA loops which, in their vector form, are not bound to the chromosome. In the present specification, "plasmid" and "vector" are used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors

which serve equivalent functions and which become known in the art subsequently hereto.

The term "wild-type allele" refers to an allele of a gene which, when present in two copies in a subject results in a wild-type phenotype. There can be several different wild-type alleles of a specific gene, since certain nucleotide changes in a gene may not affect the phenotype of a subject having two copies of the gene with the nucleotide changes.

As described below, in a preferred embodiment, the progenitor cells of the present invention are pancreatic progenitor cells. The term "pancreas" is art-recognized, and refers generally to a large, elongated, racemose gland situated transversely behind the stomach, between the spleen and duodenum. The pancreatic exocrine function, e.g., external secretion, provides a source of digestive enzymes. Indeed, "pancreatin" refers to a substance from the pancreas containing enzymes, principally amylase, protease, and lipase, which substance is used as a digestive aid. The exocrine portion is composed of several serous cells surrounding a lumen. These cells synthesize and secrete digestive enzymes such as trypsinogen, chymotrypsinogen, carboxypeptidase, ribonuclease, deoxyribonuclease, triacylglycerol lipase, phospholipase A_2, elastase, and amylase.

The endocrine portion of the pancreas is composed of the islets of Langerhans. The islets of Langerhans appear as rounded clusters of cells embedded within the exocrine pancreas. Four different types of cells- α, β, δ, and ϕ-have been identified in the islets. The α cells constitute about 20% of the cells found in pancreatic islets and produce the hormone glucagon. Glucagon acts on several tissues to make energy available in the intervals between feeding. In the liver, glucagon causes breakdown of glycogen and promotes gluconeogenesis from amino acid precursors. The δ cells produce somatostatin which acts in the pancreas to inhibit glucagon release and to decrease pancreatic exocrine secretion. The hormone pancreatic polypeptide is produced in the ϕ cells. This hormone inhibits pancreatic exocrine secretion of bicarbonate and enzymes, causes relaxation of the gallbladder, and decreases bile secretion. The most abundant cell in the islets, constituting 60-80% of the cells, is the β cell, which produces insulin. Insulin is known to cause the storage of excess nutrients arising during and shortly after feeding. The major target organs for insulin are the liver, muscle, and fat-organs specialized for storage of energy.

The term "pancreatic progenitor cell" refers to a cell which can differentiate into a cell of pancreatic lineage, e.g. a cell which can produce a hormone or enzyme normally produced by a pancreatic cell. For instance, a pancreatic progenitor cell may be caused to differentiate, at least partially, into α, β, δ, or ϕ islet cell, or a cell of exocrine fate. The pancreatic progenitor cells of the invention can also be cultured prior to administration to a subject under conditions which promote cell proliferation and differentiation. These conditions include culturing the cells to allow proliferation and confluence *in vitro* at which time the cells can be made to form pseudo islet-like aggregates or clusters and secrete insulin, glucagon, and somatostatin.

(iii) Exemplary Secretin Therapeutics

Secretin is a 27-amino acid gastrointestinal hormone that stimulates the secretion of bicarbonate-rich pancreatic fluid. Secretin is produced by specific endocrine cells, S cells, located in the mucosa of the proximal small intestine. Secretion of secretin is stimulated by the presence of either acidic pH or fatty acids in the duodenum. The amino acid sequence of secretin was determined by Mutt et al. (1970). (Gen Bank Accession Number 134405). Secretin is synthesized as a larger precursor. The nucleic acid and amino acid sequence is provided in the appended sequence listing. (SEQ ID Nos: 1 & 2).

Kopin et al. (1990) isolated cDNAs encoding the rat and porcine secretin precursors. The deduced amino acid sequence included a signal peptide, an N-terminal peptide, secretin itself, and a 72-amino acid C-terminal peptide. Secretin has been highly conserved through evolution. Rat secretin differs from its porcine counterpart by a single glutamine-for-arginine substitution at position 14. In contrast, the amino acid sequences of the C-terminal peptides are only 39% conserved between the two species, suggesting that the C-terminal peptide does not have an essential physiologic function. RNA blot hybridizations reveal that the rat secretin gene is expressed throughout the small intestine.

The subject methods can be carried out using native, purified peptide secretin or recombinant secretin, or fragments thereof, as well as peptidomimetics thereof. Homologs and analogs of secretin can be generated by mutagenesis, such as by discrete point mutation(s), or by truncation. For instance, mutation can give rise to homologs

which retain substantially the same, or merely a subset, of the biological activity of secretin. Alternatively, antagonistic forms of the protein can be generated which are able to inhibit the function of the naturally occurring form of secretin, such as by competitively binding to a cognate receptor of secretin, thus blocking signal transduction. In addition, agonistic forms of secretin may be generated which are constitutively active. Thus, secretin and homologs thereof may be either positive or negative regulators of insulin production in pancreatic islets or β cells.

Secretin analogs and mimetics may also be synthesized by many techniques that are known to those skilled in the peptide art. A summary of the many techniques available may be found in Solid Phase Peptide Synthesis 2nd ed. (Stewart, J.M. and Young, J.D., Pierce Chemical Company, Rockford, Ill. 1984). Other secretin analogs can be prepared by making appropriate modifications, within the ability of a person of ordinary skill in the art.

In general, polypeptides referred to herein as having an activity (e.g., are "bioactive") of secretin are defined as polypeptides which include an amino acid sequence corresponding (e.g., identical or homologous) to all or a portion of the amino acid sequence of secretin and which mimic or antagonize all or a portion of the biological/biochemical activities of a naturally occurring secretin protein. Such biological activity includes the induction or enhancement of differentiated function as demonstrated by induced or increased insulin production and other indicia of β cell differentiation, such as, for example, homeodomain type transcription factors such as STF-1; PAX gene(s) such as PAX6; PTF-1; hXBP-1; HNF genes(s); villin; tyrosine hydroxylase; insulin glucajon; and/or Neuropeptide Y.

The bioactivity of a secretin analog may also include the ability to alter the transcriptional rate of a gene as, for example, a downstream component of a signal transduction cascade initiated by the interaction of a secretin analog with its cognate receptor.

Other biological activities of secretin, a secretin analog or agonist are described herein or will be reasonably apparent to those skilled in the art.

A secretin polypeptide which represents a portion of the full-length polypeptide, can be either an agonist (e.g., mimics or enhances), or alternatively, an antagonist of a biological activity of a naturally occurring form of the protein, e.g., the polypeptide is

able to modulate the growth state of an insulin positive progenitor cell to authentic secretin proteins. Homologs of the subject secretin proteins include versions of the protein which are resistant to proteolytic cleavage, as for example, due to mutations which alter potential cleavage sequences or which inactivate an enzymatic activity associated with the protein.

The secretin polypeptides of the present invention which represent portions of the full-length polypeptides, can be glycosylated, or conversely, by choice of the expression system or by modification of the protein sequence to preclude glycosylation, induced carbohydrate analogs can also be provided. Glycosylated forms include derivatization with glycosaminoglycan chains.

The subject proteins can also be provided as chimeric molecules, such as in the form of fusion proteins. For instance, the secretin protein can be provided as a recombinant fusion protein which includes a second polypeptide portion, e.g., a second polypeptide having an amino acid sequence unrelated to secretin, e.g., the second polypeptide portion is glutathione-S-transferase, e.g., the second polypeptide portion is an enzymatic activity such as alkaline phosphatase, e.g. the second polypeptide portion is an epitope tag.

Analogs of secretin may emulate and enhance the duration, effect, biological activity and selectivity of the natural peptide in the treatment of pancreatic tumors (See U.S. Patent No. 5,574,010, incorporated herein by reference).

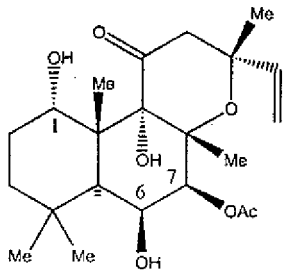
Compounds which increase the activity or expression of cAMP may be useful as secretin therapeutics, and compounds which decrease the activity or expression of cAMP may be useful as secretin antagonists. For example, compounds which may activate adenylate cyclase include forskolin (FK), cholera toxin (CT), pertussis toxin (PT), prostaglandins (e.g., PGE-1 and PGE-2), colforsin and β-adrenergic receptor agonists. β-Adrenergic receptor agonists (sometimes referred to herein as "β-adrenergic agonists") include albuterol, bambuterol, bitolterol, carbuterol, clenbuterol, clorprenaline, denopamine, dioxethedrine, dopexamine, ephedrine, epinephrine, etafedrine, ethylnorepinephrine, fenoterol, formoterol, hexoprenaline, ibopamine, isoetharine, isoproterenol, mabuterol, metaproterenol, methoxyphenamine, oxyfedrine, pirbuterol, prenalterol, procaterol, protokylol, reproterol, rimiterol, ritodrine, soterenol, salmeterol, terbutaline, tretoquinol, tulobuterol, and xamoterol.

Compounds which may inhibit a cAMP phosphodiesterase include amrinone, milrinone, xanthine, methylxanthine, anagrelide, cilostamide, medorinone, indolidan, rolipram, 3-isobutyl-1-methylxanthine (IBMX), chelerythrine, cilostazol, glucocorticoids, griseolic acid, etazolate, caffeine, indomethacin, theophylline, papverine, methyl isobutylxanthine (MIX), and fenoxamine.

Analogs of cAMP which may be useful in the present method include dibutyryl-cAMP (db-cAMP), (8-(4)-chlorophenylthio)-cAMP (cpt-cAMP), 8-[(4-bromo-2,3-dioxobutyl)thio]-cAMP, 2-[(4-bromo-2,3-dioxobutyl)thio]-cAMP, 8-bromo-cAMP, dioctanoyl-cAMP, Sp-adenosine 3':5'-cyclic phosphorothioate, 8-piperidino-cAMP, N^6-phenyl-cAMP, 8-methylamino-cAMP, 8-(6-aminohexyl)amino-cAMP, 2'-deoxy-cAMP, N^6,2'-O-dibutryl-cAMP, N^6,2'-O-disuccinyl-cAMP, N^6-monobutyryl-cAMP, 2'-O-monobutyryl-cAMP, 2'-O-monobutryl-8-bromo-cAMP, N^6-monobutryl-2'-deoxy-cAMP, and 2'-O-monosuccinyl-cAMP.

Compounds which may reduce the levels or activity of cAMP include prostaglandylinositol cyclic phosphate (cyclic PIP), endothelins (ET)-1 and -3, norepinepurine, K252a, dideoxyadenosine, melatonin, pertussis toxin, staurosporine, G_i agonists, beta-blockers, and ligands for G-protein coupled receptors. Additional compounds are disclosed in U.S. Patent Nos. 5,891,875, 5,260,210, and 5,795,756.

Above-listed compounds useful in the subject methods may be modified to increase the bioavailability, activity, or other pharmacologically relevant property of the compound. For example, forskolin has the formula:



Forskolin

Modifications of forskolin which have been found to increase the hydrophilic character of forskolin without severely attenuating the desired biological activity include acylation

of the hydroxyls at C6 and/or C7 (after removal of the acetyl group) with hydrophilic acyl groups. In compounds wherein C6 is acylated with a hydrophilic acyl group, C7 may optionally be deacylated. Suitable hydrophilic acyl groups include groups having the structure $-(CO)(CH_2)_nX$, wherein X is OH or NR_2; R is hydrogen, a C_1-C_4 alkyl group, or two Rs taken together form a ring comprising 3-8 atoms, preferably 5-7 atoms, which may include heteroatoms (e.g., piperazine or morpholine rings); and n is an integer from 1-6, preferably from 1-4, even more preferably from 1-2. Other suitable hydrophilic acyl groups include hydrophilic amino acids or derivatives thereof, such as aspartic acid, glutamic acid, asparagine, glutamine, serine, threonine, tyrosine, etc., including amino acids having a heterocyclic side chain. Forskolin, or other compounds listed above, modified by other possible hydrophilic acyl side chains known to those of skill in the art may be readily synthesized and tested for activity in the present method.

Similarly, variants or derivatives of any of the above-listed compounds may be effective as cAMP agonists in the subject method. Those skilled in the art will readily be able to synthesize and test such derivatives for suitable activity.

In certain embodiments, the subject cAMP agonists can be chosen on the basis of their selectivity for cAMP activation.

In certain embodiments, it may be advantageous to administer two or more of the above cAMP agonists, preferably of different types. For example, use of an adenylate cyclase agonist in conjunction with a cAMP phosphodiesterase antagonist may have an advantageous or synergistic effect.

In certain preferred embodiments, the subject agents raise or lower effective cAMP levels with an ED_{50} of 1 mM or less, more preferably of 1 μM or less, and even more preferably of 1 nM or less.

In certain embodiments, a secretin therapeutic may comprise a vector for transfecting a cell with a nucleic acid encoding secretin or a biologically active fragment or homolog thereof to induce a cell to recombinantly express secretin. Alternatively, a vector comprising a nucleic acid encoding a secretin-responsive receptor or a biologically active fragment or homolog thereof to induce a cell to recombinantly express the secretin-responsive receptor, such as secretin receptor. Thus, the invention further provides plasmids and vectors, which can be used to express a gene in a host cell.

The host cell may be any prokaryotic or eukaryotic cell. Thus, a nucleotide sequence derived from any one of SEQ ID Nos. 1 or 3, or a sequence complementary thereto, or encoding a protein having a sequence of one of SEQ ID Nos. 2 or 6, or a fragment, homolog, or variant thereof which retains at least one biological activity of the complete sequence, can be used to produce a recombinant form of an polypeptide via microbial or eukaryotic cellular processes. Ligating the polynucleotide sequence into a gene construct, such as an expression vector, and transforming or transfecting into hosts, either eukaryotic (yeast, avian, insect or mammalian) or prokaryotic (bacterial cells), are standard procedures well known in the art.

Vectors that allow expression of a nucleic acid in a cell are referred to as expression vectors. Typically, expression vectors contain a nucleic acid operably linked to at least one transcriptional regulatory sequence. Regulatory sequences are art-recognized and are selected to direct expression of the subject nucleic acids. Transcriptional regulatory sequences are described in Goeddel; Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). In one embodiment, the expression vector includes a recombinant gene encoding a peptide having an agonistic activity of a subject polypeptide, or alternatively, encoding a peptide which is an antagonistic form of a subject polypeptide.

The choice of plasmid will depend on the type of cell in which propagation is desired and the purpose of propagation. Certain vectors are useful for amplifying and making large amounts of the desired DNA sequence. Other vectors are suitable for expression in cells in culture. Still other vectors are suitable for transfer and expression in cells in a whole animal or person. The choice of appropriate vector is well within the skill of the art. Many such vectors are available commercially. The nucleic acid or full-length gene is inserted into a vector typically by means of DNA ligase attachment to a cleaved restriction enzyme site in the vector. Alternatively, the desired nucleotide sequence may be inserted by homologous recombination in vivo. Typically this is accomplished by attaching regions of homology to the vector on the flanks of the desired nucleotide sequence. Regions of homology are added by ligation of oligonucleotides, or by polymerase chain reaction using primers comprising both the region of homology and a portion of the desired nucleotide sequence, for example.

Nucleic acids or full-length genes are linked to regulatory sequences as appropriate to obtain the desired expression properties. These may include promoters (attached either at the 5' end of the sense strand or at the 3' end of the antisense strand), enhancers, terminators, operators, repressors, and inducers. The promoters may be regulated or constitutive. In some situations it may be desirable to use conditionally active promoters, such as tissue-specific or developmental stage-specific promoters. These are linked to the desired nucleotide sequence using the techniques described above for linkage to vectors. Any techniques known in the art may be used.

When any of the above host cells, or other appropriate host cells or organisms, are used to replicate and/or express the polynucleotides or nucleic acids of the invention, the resulting replicated nucleic acid, RNA, expressed protein or polypeptide, is within the scope of the invention as a product of the host cell or organism. The product is recovered by any appropriate means known in the art.

Once the gene corresponding to the nucleic acid is identified, its expression can be regulated in the cell to which the gene is native. For example, an endogenous gene of a cell can be regulated by an exogenous regulatory sequence as disclosed in U.S. Patent No. 5,641,670, "Protein Production and Protein Delivery."

A number of vectors exist for the expression of recombinant proteins in yeast (see, for example, Broach *et al.* (1983) in Experimental Manipulation of Gene Expression, ed. M. Inouye, Academic Press, p. 83, incorporated by reference herein). In addition, drug resistance markers such as ampicillin can be used. In an illustrative embodiment, a polypeptide is produced recombinantly utilizing an expression vector generated by sub-cloning one of the nucleic acids represented in one of SEQ ID Nos. 1 or 3, or a sequence complementary thereto.

The preferred mammalian expression vectors contain both prokaryotic sequences, to facilitate the propagation of the vector in bacteria, and one or more eukaryotic transcription units that are expressed in eukaryotic cells. The various methods employed in the preparation of plasmids and transformation of host organisms are well known in the art. For other suitable expression systems for both prokaryotic and eukaryotic cells, as well as general recombinant procedures, see Molecular Cloning: A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989) Chapters 16 and 17.

When it is desirable to express only a portion of a gene, e.g., a truncation mutant, it may be necessary to add a start codon (ATG) to the oligonucleotide fragment containing the desired sequence to be expressed. It is well known in the art that a methionine at the N-terminal position can be enzymatically cleaved by the use of the enzyme methionine aminopeptidase (MAP). MAP has been cloned from E. coli (Ben-Bassat *et al.* (1987) J. Bacteriol. 169:751-757) and Salmonella typhimurium and its *in vitro* activity has been demonstrated on recombinant proteins (Miller *et al.* (1987) PNAS 84:2718-1722). Therefore, removal of an N-terminal methionine, if desired, can be achieved either *in vivo* by expressing polypeptides in a host which produces MAP (e.g., E. coli or CM89 or S. cerevisiae), or *in vitro* by use of purified MAP (e.g., procedure of Miller *et al.*, *supra*).

The gene constructs of the present invention can also be used as part of a gene therapy protocol to deliver nucleic acids encoding either an agonistic or antagonistic form of one of the subject proteins. Thus, another aspect of the invention features expression vectors for *in vivo* or *in vitro* transfection and expression of a subject polypeptide in particular cell types so as to reconstitute the function of, or alternatively, abrogate the function of the endogenous gene in a tissue. This could be desirable, for example, when the naturally occurring form of the protein is misexpressed or the natural protein is mutated and less active.

Moreover, the nucleic acid constructs of the present invention can also be used as part of a gene therapy protocol to deliver nucleic acids such as antisense nucleic acids. Thus, another aspect of the invention features expression vectors for *in vivo* or *in vitro* transfection with an antisense oligonucleotide.

In addition to viral transfer methods, non-viral methods can also be employed to introduce a subject nucleic acid, e.g., a sequence represented by one of SEQ ID Nos. 1 or 3, or a sequence complementary thereto, into the tissue of an animal. Most nonviral methods of gene transfer rely on normal mechanisms used by mammalian cells for the uptake and intracellular transport of macromolecules. In preferred embodiments, non-viral targeting means of the present invention rely on endocytic pathways for the uptake of the subject nucleic acid by the targeted cell. Exemplary targeting means of this type include liposomal derived systems, polylysine conjugates, and artificial viral envelopes.

A nucleic acid of any of SEQ ID Nos. 1 or 3, or a sequence complementary thereto, the corresponding cDNA, or the full-length gene may be used to express the partial or complete gene product. Appropriate nucleic acid constructs are purified using standard recombinant DNA techniques as described in, for example, Sambrook *et al.*, (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed. (Cold Spring Harbor Press, Cold Spring Harbor, New York), and under current regulations described in United States Dept. of HHS, National Institute of Health (NIH) Guidelines for Recombinant DNA Research. The polypeptides encoded by the nucleic acid may be expressed in any expression system, including, for example, bacterial, yeast, insect, amphibian and mammalian systems. Suitable vectors and host cells are described in U.S. Patent No. 5,654,173.

Alternatively, secretin responsivity can be inhibited by treating a cell with an antisense nucleic acid to inhibit transcription of secretin or a secretin receptor. One aspect of the invention thus relates to the use of the isolated nucleic acid, e.g., SEQ ID Nos. 1 or 3, or a sequence complementary thereto, in antisense therapy. As used herein, antisense therapy refers to administration or *in situ* generation of oligonucleotide molecules or their derivatives which specifically hybridize (e.g., bind) under cellular conditions with the cellular mRNA and/or genomic DNA, thereby inhibiting transcription and/or translation of that gene. The binding may be by conventional base pair complementarity, or, for example, in the case of binding to DNA duplexes, through specific interactions in the major groove of the double helix. In general, antisense therapy refers to the range of techniques generally employed in the art, and includes any therapy which relies on specific binding to oligonucleotide sequences.

An antisense construct of the present invention can be delivered, for example, as an expression plasmid which, when transcribed in the cell, produces RNA which is complementary to at least a unique portion of the cellular mRNA. Alternatively, the antisense construct is an oligonucleotide probe which is generated *ex vivo* and which, when introduced into the cell, causes inhibition of expression by hybridizing with the mRNA and/or genomic sequences of a subject nucleic acid. Such oligonucleotide probes are preferably modified oligonucleotides which are resistant to endogenous nucleases, e.g., exonucleases and/or endonucleases, and are therefore stable *in vivo*. Exemplary nucleic acid molecules for use as antisense oligonucleotides are

phosphoramidate, phosphorothioate and methylphosphonate analogs of DNA (see also U.S. Patents 5,176,996; 5,264,564; and 5,256,775). Additionally, general approaches to constructing oligomers useful in antisense therapy have been reviewed, for example, by Van der Krol et al. (1988) BioTechniques 6:958-976; and Stein et al. (1988) Cancer Res 48:2659-2668. With respect to antisense DNA, oligodeoxyribonucleotides derived from the translation initiation site, e.g., between the -10 and +10 regions of the nucleotide sequence of interest, are preferred.

Antisense approaches involve the design of oligonucleotides (either DNA or RNA) that are complementary to mRNA. The antisense oligonucleotides will bind to the mRNA transcripts and prevent translation. Absolute complementarity, although preferred, is not required. In the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the mRNA, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have recently been shown to be effective at inhibiting translation of mRNAs as well. (Wagner, R. 1994. Nature 372:333). Therefore, oligonucleotides complementary to either the 5' or 3' untranslated, non-coding regions of a gene could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are typically less efficient inhibitors of translation but could also be used in accordance with the invention. Whether designed to hybridize to the 5', 3', or coding region of subject mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably less that about 100 and more preferably less than about 50, 25, 17 or 10 nucleotides in length.

Regardless of the choice of target sequence, it is preferred that *in vitro* studies are first performed to quantitate the ability of the antisense oligonucleotide to quantitate the ability of the antisense oligonucleotide to inhibit gene expression. It is preferred that these studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological effects of oligonucleotides. It is also preferred that these studies compare levels of the target RNA or protein with that of an internal control RNA or protein. Additionally, it is envisioned that results obtained using the antisense oligonucleotide are compared with those obtained using a control oligonucleotide. It is preferred that the control oligonucleotide is of approximately the same length as the test oligonucleotide and that the nucleotide sequence of the oligonucleotide differs from the antisense sequence no more than is necessary to prevent specific hybridization to the target sequence.

The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO 88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication No. WO 89/10134, published April 25, 1988), hybridization-triggered cleavage agents (See, e.g., Krol et al., 1988, BioTechniques 6:958-976), or intercalating agents (See, e.g., Zon, 1988, Pharm. Res. 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is preferably selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxytriethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine,

2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5- oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety, preferably selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose.

The antisense oligonucleotide can also contain a neutral peptide-like backbone. Such molecules are termed peptide nucleic acid (PNA)-oligomers and are described, e.g., in Perry-O'Keefe et al. (1996) Proc. Natl. Acad. Sci. U.S.A. 93:14670 and in Eglom *et al.* (1993) Nature 365:566. One advantage of PNA oligomers is their capability to bind to complementary DNA essentially independently from the ionic strength of the medium due to the neutral backbone of the DNA. In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet a further embodiment, the antisense oligonucleotide is an α-anomeric oligonucleotide. An α-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641). The oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-12148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

Oligonucleotides of the invention may be synthesized by standard methods known in the art, e.g., by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al.

(1988, Nucl. Acids Res. 16:3209), methylphosphonate olgonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

While antisense nucleotides complementary to a coding region sequence can be used, those complementary to the transcribed untranslated region and to the region comprising the initiating methionine are most preferred.

The antisense molecules can be delivered to cells which express the target nucleic acid *in vivo*. A number of methods have been developed for delivering antisense DNA or RNA to cells; e.g., antisense molecules can be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

However, it is often difficult to achieve intracellular concentrations of the antisense sufficient to suppress translation on endogenous mRNAs. Therefore, a preferred approach utilizes a recombinant DNA construct in which the antisense oligonucleotide is placed under the control of a strong pol III or pol II promoter. The use of such a construct to transfect target cells in the patient will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous transcripts and thereby prevent translation of the target mRNA. For example, a vector can be introduced *in vivo* such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto *et al.*, 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al, 1982, Nature 296:39-

42), etc. Any type of plasmid, cosmid, YAC or viral vector can be used to prepare the recombinant DNA construct which can be introduced directly into the tissue site; e.g., the choroid plexus or hypothalamus. Alternatively, viral vectors can be used which selectively infect the desired tissue (e.g., for brain, herpesvirus vectors may be used), in which case administration may be accomplished by another route (e.g., systemically).

In another aspect of the invention, ribozyme molecules designed to catalytically cleave target mRNA transcripts can be used to prevent translation of target mRNA and expression of a target protein (See, e.g., PCT International Publication WO90/11754, published October 4, 1990; Sarver *et al.*, 1990, Science 247:1222-1225 and U.S. Patent No. 5,093,246). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy target mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, 1988, Nature, 334:585-591. Preferably the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the target mRNA; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one which occurs naturally in *Tetrahymena thermophila* (known as the IVS, or L-19 IVS RNA) and which has been extensively described by Thomas Cech and collaborators (Zaug, et al., 1984, Science, 224:574-578; Zaug and Cech, 1986, Science, 231:470-475; Zaug, et al., 1986, Nature, 324:429-433; published International patent application No. WO88/04300 by University Patents Inc.; Been and Cech, 1986, Cell, 47:207-216). The Cech-type ribozymes have an eight base pair active site which hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place. The invention encompasses those Cech-type ribozymes which target eight base-pair active site sequences that are present in a target gene.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (e.g., for improved stability, targeting, etc.) and should be delivered to

cells which express the target gene *in vivo*. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous messages and inhibit translation. Because ribozymes, unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Antisense RNA, DNA, and ribozyme molecules of the invention may be prepared by any method known in the art for the synthesis of DNA and RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides and oligoribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

Endogenous gene expression can also be reduced by inactivating or "knocking out" the gene or its promoter using targeted homologous recombination. (E.g., see Smithies *et al.*, 1985, Nature 317:230-234; Thomas & Capecchi, 1987, Cell 51:503-512; Thompson et al., 1989 Cell 5:313-321; each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional gene (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous gene (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express that gene *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the gene.

Alternatively, endogenous gene expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the target gene (i.e., the gene promoter and/or enhancers) to form triple helical structures that prevent transcription of the gene in target cells in the body. (See generally, Helene, C.

1991, Anticancer Drug Des., 6(6):569-84; Helene, C., et al., 1992, Ann. N.Y. Accad. Sci., 660:27-36; and Maher, L.J., 1992, Bioassays 14(12):807-15).

Nucleic acid molecules to be used in triple helix formation for the inhibition of transcription are preferably single stranded and composed of deoxyribonucleotides. The base composition of these oligonucleotides should promote triple helix formation via Hoogsteen base-pairing rules, which generally require sizable stretches of either purines or pyrimidines to be present on one strand of a duplex. Nucleotide sequences may be pyrimidine-based, which will result in TAT and CGC triplets across the three associated strands of the resulting triple helix. The pyrimidine-rich molecules provide base complementarity to a purine-rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, nucleic acid molecules may be chosen that are purine-rich, for example, containing a stretch of G residues. These molecules will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand of the targeted duplex, resulting in CGC triplets across the three strands in the triplex.

Alternatively, the potential sequences that can be targeted for triple helix formation may be increased by creating a so called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizable stretch of either purines or pyrimidines to be present on one strand of a duplex.

Antisense RNA and DNA, ribozyme, and triple helix molecules of the invention may be prepared by any method known in the art for the synthesis of DNA and RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides and oligoribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

Moreover, various well known modifications to nucleic acid molecules may be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of flanking sequences of ribonucleotides or deoxyribonucleotides to the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the oligodeoxyribonucleotide backbone.

(iv) Exemplary Uses of Secretin Therapeutics

A salient feature of the subject method concerns the use of defined explants as sources from which discrete progenitor cell populations can be amplified. Moreover, the subject method generally does not require much starting material. Accordingly, small samples of pancreatic tissue from a donor can be obtained without sacrificing or seriously injuring the donor. The progenitor cells can be amplified, and subsequently isolated from the epithelial explant, and treated with the secretin therapeutics of the present invention.

There are a large number of tissue culture media that exist for culturing tissue from animals. Some of these are complex and some are simple. While it is expected that the ductal epithelial explants may grow in complex media, it will generally be preferred that the explants be maintained in a simple medium, such as Dulbecco's Minimal Essential Media (DMEM), in order to effect more precise control over the activation of certain progenitor populations in the explant. In a preferred embodiment, the pancreatic ductal epithelium is cultured in Isocave modified MEM cell culture medium with 5% FBS. Moreover, the explants can be maintained in the absence of sera for extended periods of time. In preferred embodiments of the invention, the growth factors or other mitogenic agents are not included in the primary media for maintenance of the cultures *in vitro*, but are used subsequently to cause proliferation of distinct populations of progenitor cells.

The cultures may be maintained in any suitable culture vessel, such as a 12 or 24 well microplate, and may be maintained under typical culture conditions for cells isolated from the same animal, e.g., such as 37 °C in 5% CO_2. The cultures may be shaken for improved aeration, the speed of shaking being, for example, 12 rpm.

In order to isolate progenitor cells from the ductal cultures, it will generally be desirable to contact the explant with an agent, such as the secretin therapeutics of the instant invention, which causes proliferation of one or more populations of progenitor cells in the explant. For instance, a secretin agonist, e.g., a substance that induces mitosis and cell transformation, can be used to detect a progenitor cell population in the explant, and where desirable, to cause the amplification of that population. To illustrate, a purified or semi-purifed preparation of a growth factor can be applied to the culture. Induction of progenitor cells which respond to the applied secretin therapeutic can be detected by proliferation of the progenitor cells. However, as described below, amplification of the population need not occur to a large extent in order to use certain techniques for isolating the responsive population.

In yet other embodiments, the ductal explants and/or amplified progenitor cells can be cultured on feeder layers, e.g., layers of feeder cells which secrete inductive factors or polymeric layers containing inductive factors. For example, a matrigel layer can be used to induce hematopoietic progenitor cell expansion, as described in the appended examples. Matrigel (Collaborative Research, Inc., Bedford, Mass.) is a complex mixture of matrix and associated materials derived as an extract of murine basement membrane proteins, consisting predominantly of laminin, collagen IV, heparin sulfate proteoglycan, and nidogen and entactin was prepared from the EHS tumor as described Kleinman et al, "Basement Membrane Complexes with Biological Activity", Biochemistry, Vol. 25 (1986), pages 312-318. Other such matrixes can be provided, such as Humatrix. Likewise, natural and recombinantly engineered cells can be provided as feeder layers to the instant cultures.

Methods of measuring cell proliferation are well known in the art and most commonly include determining DNA synthesis characteristic of cell replication. There are numerous methods in the art for measuring DNA synthesis, any of which may be used according to the invention. In an embodiment of the invention, DNA synthesis has been determined using a radioactive label
(^{3}H-thymidine) or labeled nucleotide analogues (BrdU) for detection by immunofluorescence.

However, in addition to measuring DNA synthesis, morphological changes can be, and preferably will be, relied on as the basis for isolating responsive progenitor cell

populations. For instance, as described in the appended examples, we have observed that certain growth factors cause amplification of progenitor cells in ductal explants so as to form structures that can be easily detected by the naked eye or microscopy. In an exemplary embodiment, those progenitor cells which respond to growth factors by proliferation and subsequent formation of outgrowths from the explant, e.g., buds or blebs, can be easily detected. In another illustrative embodiment, other structural changes, e.g., changes in optical density of proliferating cells, can be detected via contrast microscopy.

Various techniques may be employed to isolate the activated progenitor cells of treated explant. Preferred isolation procedures for progenitor cells are the ones that result in as little cell death as possible. For example, the activated progenitor cells can be removed from the explant sample by mechanical means, e.g., mechanically sheared off with a pipette. In other instances, it will be possible to dissociate the progenitor cells from the entire explant, or sub-portion thereof, e.g., by enzymatic digestion of the explant, followed by isolation of the activated progenitor cell population based on specific cellular markers, e.g., using affinity separation techniques or fluorescence activated cell sorting (FACS).

To further illustrate, the examples below demonstrate that ductal explants contain growth factor-responsive progenitor cell types. It is further demonstrated that different growth factors can induce/amplify distinct populations of progenitor cells within the ductal tissue explant to proliferate. This indicates the presence of specific growth factor receptors on the surface of distinct progenitor cell populations. This is important because the expression of these receptors marks the progenitor cell populations of interest. For example, cells responsive to the introduction of secretin, e.g., by differentiating, can be identified and isolated. Monoclonal antibodies are particularly useful for identifying markers (surface membrane proteins, e.g., receptors) associated with particular cell lineages and/or stages of differentiation. Thus, for example, cells bearing receptors responsive to secretin, e.g., secretin receptor, may be identified and isolated. Procedures for separation of the subject progenitor cell may include magnetic separation, using antibody-coated magnetic beads, affinity chromatography, and "panning" with antibody attached to a solid matrix, e.g., plate, or other convenient technique. Techniques providing accurate separation include fluorescence-activated cell

sorting, which can have varying degrees of sophistication, e.g., a plurality of color channels, low angle and obtuse light scattering detecting channels, impedance channels, etc.

Conveniently, the antibodies may be conjugated with markers, such as magnetic beads, which allow for direct separation, biotin, which can be removed with avidin or streptavidin bound to a support, fluorochromes, which can be used with a fluorescence-activated cell sorter, or the like, to allow for ease of separation of the particular cell type. Any technique may be employed which is not unduly detrimental to the viability of the cells.

In an illustrative embodiment, some of the antibodies for growth factor receptors that exist on the subject progenitor cells are commercially available (e.g., antibodies for EGF receptors, FGF receptors and/or TGF receptors), and for other growth factor receptors, antibodies can be made by methods well known to one skilled in the art. In addition to using antibodies to isolate progenitor cells of interest, one skilled in the art can also use the growth factors themselves to label the cells, for example, to permit "panning" processes.

Upon isolation, the progenitor cells of the present invention can be further characterized in the following manner: responsiveness to growth factors, specific gene expression, antigenic markers on the surface of such cells, and/or basic morphology.

For example, extent of growth factor responsivity, e.g., the concentration range of growth factor to which they will respond to, the maximal and minimal responses, and to what other growth factors and conditions to which they might respond, can be used to characterize the subject progenitor cells. Furthermore, the isolated progenitor cells can be characterized by the expression of genes known to mark the developing (i.e., stem or progenitor) cells for the pancreas.

In an illustrative embodiment, the hepatocyte nuclear factor (HNF) transcription factor family, e.g., HNF1-4, are known to be expressed in various cell types at various times during pancreas development. For example, the progenitor cell may express one or more HNF protein such as HNF1α, HNF1β, HNF3β, HNF3γ, and/or HNF4. Glut2 is also a marker for both early pancreatic cells. Certain of the "forkhead" transcription factors, such as fkh-1 or the like, are understood to be markers in early gut tissue.

In another illustrative embodiment, homeodomain type transcription factors such as STF-1 (also known as IPF-1, IDX-1 or PDX) have recently been shown to mark different populations of the developing pancreas. Some LIM genes have also been shown to regulate insulin gene expression and would also be markers for protodifferentiated β islet cells. Likewise, certain of the PAX genes, such as PAX6, are expressed during pancreas formation and may be used to characterize certain pancreatic progenitor cell populations. Other markers of pancreatic progenitor cells include the pancreas specific transcription factor PTF-1, and hXBP-1 and the like. Moreover, certain of the HNF proteins are expressed during early pancrease development and may used as markers for pancreatic progenitor cells.

Progenitor cells giving rise to pancreatic cells may also express such as markers as villin and/or tyrosine hydroxylase, as well as secrete such factors as insulin, glucagon and/or neuropeptide Y.

Once isolated and characterized, the subject progenitor cells can be cultured under conditions which allow further differentiation into specific cell lineages. This can be achieved through a paradigm of induction that can be developed. For example, the subject progenitor cells can be recombined with the corresponding embryonic tissue to see if the embryonic tissue can instruct the adult cells to codevelop and codifferentiate. Alternatively, the progenitor cells can be contacted with one or more growth or differentiation factors which can induce differentiation of the cells. For instance, the cells can be treated with an agent such as forskolin, dibutyryl cAMP, Na-butyrate, dexamethasone, cholera toxin, or a growth factor such as TGFβ or a DVR sub-family member.

In another preferred embodiment, the subject progenitor cells can be implanted into one of a number of regeneration models used in the art, e.g., a host animal which has undergone partial pancreatectomy or streptozocin treatment of a host animal.

Accordingly, another aspect of the present invention pertains to the progeny of the subject progenitor cells, e.g. those cells which have been derived from the cells of the initial explant culture. Such progeny can include subsequent generations of progenitor cells, as well as lineage-committed cells generated by inducing differentiation of the subject progenitor cells after their isolation from the explant, e.g., induced *in vitro*.

Yet another aspect of the present invention concerns cellular compositions which include, as a cellular component, substantially pure preparations of the subject progenitor cells, or the progeny thereof. Cellular compositions of the present invention include not only substantially pure populations of the progenitor cells, but can also include cell culture components, e.g., culture media including amino acids, metals, coenzyme factors, as well as small populations of non-progenitor cells, e.g, some of which may arise by subsequent differentiation of isolated progenitor cells of the invention. Furthermore, other non-cellular components include those which render the cellular component suitable for support under particular circumstances, e.g., implantation, e.g., continuous culture.

As common methods of administering the progenitor cells of the present invention to subjects, particularly human subjects, which are described in detail herein, include injection or implantation of the cells into target sites in the subjects, the cells of the invention can be inserted into a delivery device which facilitates introduction by, injection or implantation, of the cells into the subjects. Such delivery devices include tubes, e.g., catheters, for injecting cells and fluids into the body of a recipient subject. In a preferred embodiment, the tubes additionally have a needle, e.g., a syringe, through which the cells of the invention can be introduced into the subject at a desired location. The progenitor cells of the invention can be inserted into such a delivery device, e.g., a syringe, in different forms. For example, the cells can be suspended in a solution or embedded in a support matrix when contained in such a delivery device. As used herein, the term "solution" includes a pharmaceutically acceptable carrier or diluent in which the cells of the invention remain viable. Pharmaceutically acceptable carriers and diluents include saline, aqueous buffer solutions, solvents and/or dispersion media. The use of such carriers and diluents is well known in the art. The solution is preferably sterile and fluid to the extent that easy syringability exists. Preferably, the solution is stable under the conditions of manufacture and storage and preserved against the contaminating action of microorganisms such as bacteria and fungi through the use of, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. Solutions of the invention can be prepared by incorporating progenitor cells as described herein in a pharmaceutically acceptable carrier or diluent and, as required, other ingredients enumerated above, followed by filtered sterilization.

Support matrices in which the progenitor cells can be incorporated or embedded include matrices which are recipient-compatible and which degrade into products which are not harmful to the recipient. Natural and/or synthetic biodegradable matrices are examples of such matrices. Natural biodegradable matrices include plasma clots, e.g., derived from a mammal, and collagen matrices. Synthetic biodegradable matrices include synthetic polymers such as polyanhydrides, polyorthoesters, and polylactic acid. Other examples of synthetic polymers and methods of incorporating or embedding cells into these matrices are known in the art. *See e.g.*, U.S. Patent No. 4,298,002 and U.S. Patent No. 5,308,701. These matrices provide support and protection for the fragile progenitor cells *in vivo* and are, therefore, the preferred form in which the progenitor cells are introduced into the recipient subjects.

The present invention also provides substantially pure progenitor cells which can be used therapeutically for treatment of various disorders associated with insufficient functioning of the pancreas.

To illustrate, the subject progenitor cells can be used in the treatment of a variety of pancreatic disorders, both exocrine and endocrine. For instance, the progenitor cells can be used to produce populations of differentiated pancreatic cells for repair subsequent to partial pancreatectomy, e.g., excision of a portion of the pancreas. Likewise, such cell populations can be used to regenerate or replace pancreatic tissue loss due to, pancreatolysis, e.g., destruction of pancreatic tissue, such as pancreatitis, e.g., a condition due to autolysis of pancreatic tissue caused by escape of enzymes into the substance.

In an exemplary embodiment, the subject progenitor cells can be provided for patients suffering from any insulin-deficiency disorder. For instance, each year, over 728,000 new cases of diabetes are diagnosed and 150,000 Americans die from the disease and its complications; the total yearly cost in the United States is over 20 billion dollars (Langer et al. (1993) *Science* 260:920-926). Diabetes is characterized by pancreatic islet destruction or dysfunction leading to loss of glucose control. Diabetes mellitus is a metabolic disorder defined by the presence of chronically elevated levels of blood glucose (hyperglycemia). Insulin-dependent (Type 1) diabetes mellitus ("IDDM") results from an autoimmune-mediated destruction of the pancreatic β-cells with consequent loss of insulin production, which results in hyperglycemia. Type 1 diabetics

require insulin replacement therapy to ensure survival. Non-insulin-dependent (Type 2) diabetes mellitus ("NIDDM") is initially characterized by hyperglycemia in the presence of higher-than-normal levels of plasma insulin (hyperinsulinemia). In Type 2 diabetes, tissue processes which control carbohydrate metabolism are believed to have decreased sensitivity to insulin. Progression of the Type 2 diabetic state is associated with increasing concentrations of blood glucose, and coupled with a relative decrease in the rate of glucose-induced insulin secretion.

The primary aim of treatment in both forms of diabetes mellitus is the same, namely, the reduction of blood glucose levels to as near normal as possible. Treatment of Type 1 diabetes involves administration of replacement doses of insulin. In contrast, treatment of Type 2 diabetes frequently does not require administration of insulin. For example, initial therapy of Type 2 diabetes may be based on diet and lifestyle changes augmented by therapy with oral hypoglycemic agents such as sulfonylurea. Insulin therapy may be required, however, especially in the later stages of the disease, to produce control of hyperglycemia in an attempt to minimize complications of the disease, which may arise from islet exhaustion.

More recently, tissue-engineering approaches to treatment have focused on transplanting healthy pancreatic islets, usually encapsulated in a membrane to avoid immune rejection. Three general approaches have been tested in animal models. In the first, a tubular membrane is coiled in a housing that contained islets. The membrane is connected to a polymer graph that in turn connects the device to blood vessels. By manipulation of the membrane permeability, so as to allow free diffusion of glucose and insulin back and forth through the membrane, yet block passage of antibodies and lymphocytes, normoglycemia was maintained in pancreatectomized animals treated with this device (Sullivan et al. (1991) *Science* 252:718).

In a second approach, hollow fibers containing islet cells were immobilized in the polysaccharide alginate. When the device was place intraperitoneally in diabetic animals, blood glucose levels were lowered and good tissue compatibility was observed (Lacey et al. (1991) *Science* 254:1782).

Finally, islets have been placed in microcapsules composed of alginate or polyacrylates. In some cases, animals treated with these microcapsules maintained normoglycemia for over two years (Lim et al. (1980) *Science* 210:908; O'Shea et al.

(1984) *Biochim. Biochys. Acta.* 840:133; Sugamori et al. (1989) *Trans. Am. Soc. Artif. Intern. Organs* 35:791; Levesque et al. (1992) *Endocrinology* 130:644; and Lim et al. (1992) *Transplantation* 53:1180). However, all of these transplantation strategies require a large, reliable source of donor islets.

The pancreatic progenitor cells of the invention can be used for treatment of diabetes because they have the ability to differentiate into cells of pancreatic lineage, e.g., β islet cells. The progenitor cells of the invention can be cultured *in vitro* under conditions which can further induce these cells to differentiate into mature pancreatic cells, or they can undergo differentiation in vivo once introduced into a subject. Many methods for encapsulating cells are known in the art. For example, a source of β islet cells producing insulin is encapsulated in implantable hollow fibers. Such fibers can be pre-spun and subsequently loaded with the β islet cells (Aebischer et al. U.S. Patent No. 4,892,538; Aebischer et al. U.S. Patent No. 5,106,627; Hoffman et al. (1990) *Expt. Neurobiol.* 110:39-44; Jaeger et al. (1990) *Prog. Brain Res.* 82:41-46; and Aebischer et al. (1991) *J. Biomech. Eng.* 113:178-183), or can be co-extruded with a polymer which acts to form a polymeric coat about the β islet cells (Lim U.S. Patent No. 4,391,909; Sefton U.S. Patent No. 4,353,888; Sugamori et al. (1989) *Trans. Am. Artif. Intern. Organs* 35:791-799; Sefton et al. (1987) *Biotehnol. Bioeng.* 29:1135-1143; and Aebischer et al. (1991) *Biomaterials* 12:50-55).

Moreover, in addition to providing a source of implantable cells, either in the form of the progenitor cell population of the differentiated progeny thereof, the subject cells can be used to produce cultures of pancreatic cells for production and purification of secreted factors. For instance, cultured cells can be provided as a source of insulin. Likewise, exocrine cultures can be provided as a source for pancreatin.

(v) Exemplary Assays

Yet another aspect of the present invention provides methods for screening various compounds for their ability to modulate growth, proliferation or differentiation of distinct progenitor cell populations from pancreatic ductal epithelial culture. In an illustrative embodiment, the subject progenitor cells, and their progeny, can be used to screen various compounds or natural products. Such explants can be maintained in minimal culture media

for extended periods of time (e.g., for 21 days or longer) and can be contacted with any compound, e.g., small molecule or natural product, e.g., growth factor, to determine the effect of such compound on one of cellular growth, proliferation or differentiation of progenitor cells in the explant. Detection and quantification of growth, proliferation or differentiation of these cells in response to a given compound provides a means for determining the compound's efficacy at inducing one of the growth, proliferation or differentiation in a given ductal explant. Methods of measuring cell proliferation are well known in the art and most commonly include determining DNA synthesis characteristic of cell replication. There are numerous methods in the art for measuring DNA synthesis, any of which may be used according to the invention. In an embodiment of the invention, DNA synthesis has been determined using a radioactive label (^{3}H-thymidine) or labeled nucleotide analogues (BrdU) for detection by immunofluorescence. The efficacy of the compound can be assessed by generating dose response curves from data obtained using various concentrations of the compound. A control assay can also be performed to provide a baseline for comparison. Identification of the progenitor cell population(s) amplified in response to a given test agent can be carried out according to phenotyping.

In one embodiment, the invention provides methods for the identification and isolation of insulin positive progenitor cells. For example, in one embodiment this invention provides a method for isolating progenitor cells. For instance, insulin-positive progenitor cells may be characterized by their expression of a secretin-responsive receptor. Antibodies reactive with secretin-responsive receptor polypeptides or with an amino acid sequence at least 60%, preferably at least 70%, even more preferably at least 80%, 90%, or 95% identical to a secretin-responsive receptor, such as SEQ ID No. 4, can thus be used to identify and purify secretin-responsive progenitor cells from the remainder of a ductal pancreatic epithelial cell population. A number of methods for sorting cell populations based upon their expression of a specific cell-surface antigen have been described. These methods can be divided into two major groups: (1) bulk sorters and (2) single-cell-based sorters. This latter group mainly refers to fluorescence-activated cell sorting (FACS) by flow cytometry (FCM). In both cases, separation of cell subsets is based on their classification according to one or more cell characteristics.

In bulk sorters, cell classification and sorting are usually achieved in a single step; by contrast, in FACS techniques, these two steps are independent sequential processes. In

addition, bulk sorters generally use a single-cell characteristic to isolate cell subsets and have a higher throughput rate, as compared with FACS by FCM, where several parameters can be used simultaneously to classify cells for their further isolation. As a consequence of the mechanisms underlying these two cell sorting methods, the balance between cell purity and cell recovery on the sorted fraction are generally different, the single-cell-based methods usually providing both a higher purity and recovery. Thus, in practice, bulk separation methods are frequently used either as a preparative step for FCM-based cell sorting or for the enrichment of the sample in specific cell subsets, when a higher throughput rate is required; in contrast, FACS by FCM is selected for the isolation of cell subsets when a high purity and, especially, recovery of a specific subpopulation of cells present in a sample are needed. Applying these concepts to the immediate invention, FACS flow cytometry technology can be employed using appropriate fluorescent marker-tagged anti-secretin receptor antibodies to effect the separation of secretin receptor-expressing progenitor cells from a heterogeneous cell population (see also the FACS flow cytometry examples described below).

For example, insulin-positive progenitor cells can be identified from a population of ductal pancreatic epithelial cells by screening for alterations in cellular growth state in response to the administration of secretin. Specifically, a population of ductal pancreatic epithelial cells is seeded onto a microtiter plate utilizing limit dilution techniques so as to yield an average of one or more cells per well. The individual isolated cells are then screened for their ability to undergo alterations in their growth state in response to the administration of secretin. In one aspect, alteration in the growth state is monitored by counting the number of cells present in each well after administration of secretin and comparing to the number of cells present in each well before administration of secretin. Microtiter wells in which the greatest expansion of the cell population is seen are those which were initially seeded with insulin-positive progenitor cells. The insulin-positive progenitor cells so identified can then be isolated from the remainder of the ductal pancreatic epithelial cell population and expanded or otherwise manipulated. In another aspect, alterations in the growth state is monitored by observing the number of phenotypic floater cells in each microtiter well before and after administration of secretin. The formation and/or expansion in the number of floater cells in a particular microtiter well in

response to secretin administration provides another indication of the presence of insulin-positive progenitor cells in that particular microtiter well cell population.

In yet another aspect, alteration in the growth state is monitored by the formation of insulin in response to the administration of secretin. For example, the presence of immunologically reactive insulin in the media of the aforementioned microtiter wells can be monitored both before and after the administration of secretin. An increase in the amount of immunologically reactive insulin in a particular well following secretin administration is indicative of the presence of insulin positive progenitor cells in that particular microtiter well cell population. The insulin-positive progenitor cells so identified can then be isolated from the remainder of the ductal pancreatic epithelial cell population and expanded or otherwise utilized as suggested below.

A similar separation of cells mitotically or otherwise phenotypically responsive to secretin can also be achieved by using appropriately adapted FACS flow cytometry technology as outlined below. Such techniques facilitate the automated identification and isolation of large numbers of insulin-positive progenitor cells.

In another embodiment, the invention provides assays for the identification of compounds that induce or modulate the growth state of insulin-positive progenitor cells. For example, the insulin-positive progenitor cells identified by the abovementioned methodology can be utilized in subsequent assays to screen for compounds which induce their growth state in a secretin-independent manner. Specifically, an isolated population of insulin-positive progenitor cells may be seeded onto a microtiter plate utilizing limit dilution techniques so as to yield an average of one or more cells per well. The isolated progenitor cell populations may subsequently be exposed individually to various candidate compounds. The ability of specific candidate compounds to induce alterations in the growth state of the isolated progenitor cell population provides a convenient screen for therapeutic agents that, like secretin, potentiate the expansion and differentiation of this important pancreatic cell type. In various versions of this embodiment of the invention, the aspect of the growth state that is monitored in the screen is one or more of the following: mitotic growth state (e.g., cell expansion), cell phenotypic state (i.e. the presence and number of floater cells), and marker gene expression state (e.g., the expression of insulin). Compounds which test positive in the abovementioned assays are useful both directly and indirectly for various therapeutic applications of the invention. For example, such

compounds may be useful for the in vitro culturing of pancreatic cell aggregates and organoids.

In still another embodiment, the invention provides assays for the identification of secretin antagonists. For example, the insulin positive progenitor cells identified by the abovementioned methodology can be utilized in subsequent assays to screen for compounds which antagonize the ability of secretin to induce the growth state of insulin-positive progenitor cells. Specifically, an isolated population of insulin-positive progenitor cells may be seeded onto a microtiter plate utilizing limit dilution techniques so as to yield an average of one or more cells per well. The isolated progenitor cell populations may subsequently be exposed to a particular amount of secretin which is found to be sufficient to induce various aspects of progenitor cell growth state as outlined above. Various candidate compounds may then be individually added to individual wells containing the secretin-induced insulin-positive progenitor cells. The ability of specific candidate compounds to prevent secretin-induced alterations in growth state of the isolated progenitor cell population provides a convenient screen for therapeutic agents that antagonize the action of secretin. In various versions of this embodiment of the invention, the aspect of the growth state that is monitored in the screen is one or more of the following: mitotic growth state (e.g., cell expansion), cell phenotypic state (i.e. the presence and number of floater cells), and marker gene expression state (e.g., the expression of insulin). Compounds which test positive in the abovementioned assays are useful both directly and indirectly for various therapeutic applications of the invention. For example, the ability to antagonize secretin signaling may allow experimental manipulation of the processes leading to pancreatic beta cell differentiation.

In yet another embodiment, the invention provides assays for the identification of compounds that antagonize insulin inhibition of secretin induction. For example, the insulin-positive progenitor cells identified by the abovementioned methodology can be utilized in subsequent assays to screen for compounds which antagonize the ability of insulin to block the ability of secretin to induce the growth state of insulin positive progenitor cells. Specifically, an isolated population of insulin-positive progenitor cells may be seeded onto a microtiter plate utilizing limit dilution techniques so as to yield an average of one or more cells per well. The isolated progenitor cell populations may subsequently be exposed to a particular amount of secretin which is found to be sufficient to induce

various aspects of progenitor cell growth state as outlined above. Next a particular amount of insulin may be added to each of the individual progenitor cell populations so as to block secretin-induced alterations of progenitor cell growth state. Various candidate compounds may then be individually added to individual wells containing the insulin-blocked, secretin-induced positive progenitor cells. The ability of specific candidate compounds to prevent inhibition by insulin of secretin-induced growth state alterations provides a convenient screen for therapeutic agents that antagonize the inhibitory action of insulin upon secretin. In various versions of this embodiment of the invention, the aspect of the growth state that is monitored in the screen is one or more of the following: mitotic growth state (e.g., cell expansion), cell phenotypic state (i.e. the presence and number of floater cells), and marker gene expression state (e.g., the expression of insulin). Compounds which test positive in the abovementioned assays are useful both directly and indirectly for various therapeutic applications of the invention. For example, the ability to antagonize the inhibitory action of insulin upon secretin signaling may allow the in situ manipulation of insulin-positive progenitor cells formation.

As for the identification and isolation of insulin-positive progenitor cells using FACS flow cytometry, the various screening assays provided by the invention can be adapted to this high throughput technology. Examples of various applications of FACS technology that can be adapted to the method of the invention are outlined below.

Examples employing FACS cell sorting

The flow cytometer is a laser based optical instrument that is able to analyze a population of cells for a variety of biochemical parameters in seconds. For example, the Becton-Dickinson FACS Vantage Flow Cytometer (FACS) is a suitable instrument. The FACS is fitted with two large coherent lasers making activation of fluorochromes from UV to far red possible. It is capable of eight parameter analyses including detection of five different fluorochromes in combination with forward and side scatter. Forward and side scatter are used to determine the size and granularity of cells. The FACS is also able to sort cells at rates up to 5,000-7,000 cells/sec and is equipped with an automated cell deposition unit that can place selected cells into 96 well tissue culture plates. A separate computer workstation for data analysis is possible. There are multiple uses of the FACS of which a sampling is offered below.

I. Analysis

Cell Cycle Analysis: Shifts in the cell cycle status of a population created by drugs, mutations, oncogenes, growth factors, hormones, nutrients, etc., can readily be assessed on the FACS using DNA analysis to determine the proportion of cells that are in Go/G1, S or G2/M phase. The FACS can analyze 10,000 cells for cell cycle distribution in about a minute so that shifts in cycling status can readily be discerned.

Apoptosis: Extensions of cell cycle analysis make it possible to rapidly determine the fraction of cells within a population undergoing programmed cell death with >95% accuracy. Simple DNA binding dyes such as propidium iodide, acridine orange, mithramycin, Hoechst, DAPI, etc., can be used (Cytometry 13:137, 1992) avoiding the costs and tedium of kits.

Ploidy: Similar methodology is used to evaluate tumor cells and cell lines for abnormal number of chromosomes.

Ca++ Release: Changes in intracellular Ca++ fluxes can readily be measured on the FACS to assess Ca++ fluxes in the cell due to signaling, state of activation of the cells, degree of viability, etc.

Mitochondrial Activity: Fluorochromes such as Rhodamine 123 are available that makes it possible to compare mitochondrial activity among groups of cells.

Expression of Cell Surface Markers, Receptors, Proteins or Enzymes: One of the most valuable uses of FACS is the evaluation of expression of cell surface markers using specific fluorochrome labeled antibodies directed at the molecules of interest. A large array of markers (CD) are used by immunologists to assess for the proportion of T-cells, B-cells, granulocytes, etc., in a population of cells. However, expression and distribution of transport proteins, hormone receptors, adhesion proteins, enzymes, etc., on the surface of cells can also readily be evaluated by flow cytometry. Here again, 10,000 cells can be assessed for these parameters in minutes using the FACS.

Detection of Oncogenes, Enzymes, Growth Factors, etc.: Permeabilization of cells in combination with appropriate fluorochrome labeled antibodies now makes it possible to detect and quantitate proteins more internal to the cell such as oncogenes, cytokines, cytoplasmic receptors, enzymes, etc.

II. Combinatorial Analysis

Perhaps the greatest strength of FACS is its ability to rapidly assess individual cells for multiple biochemical parameters. For example, it is possible to combine DNA analysis with quantitation of cell surface markers to observe changes in expression of receptors, CD markers, enzymes, etc., with changes in cell cycle status. Changes in expression these molecules as cells enter an apoptotic state can also be evaluated. Likewise, one can measure the change in expression of membrane receptors, adhesion molecules, or CD markers as the cell differentiates, becomes transformed, etc. For example, a typical side and forward scatter profile used to identify major populations of cells in the blood can be achieved as follows. As cells pass through the laser beam the light is changed based on the size (forward scatter) and granularity (side scatter) of each cell. In this manner the large and very granular neutrophils (polys) can readily be differentiated from the smaller less granular lymphocytes in the blood. Indeed, this sort of profile is used to gate or analyze specific subsets of cells such as lymphocytes within a heterogenous population. Such a profile can be used to gate or focus on a specific group of cells such as lymphocytes when further analysis is desired. For example, fluoresceinated antibodies against IgM and CD43 can be used along with scatter profiles to discern the distribution of early B-cells in the bone marrow. The latter is a very heterogenous population, yet it was possible using these tools to focus on cells that were only a few percent of the marrow. For example, fluorochrome tagged antibodies against IgM and CD43 along with forward and side scatter could be used to determine the size and distribution of early precursor B-cells in the marrow (four parameter analysis).

III. Cell Sorting

The FACS also provides the powerful ability to select and purify a specific subset of cells within a population for further study. Cells in a particular phase of the cell cycle such as M phase, cells expressing high levels of a receptor, cells devoid of a particular phosphatase or oncogene, etc., can be selected from a population and sorted into a tube or cell culture plate using the FACS. Another growing use of the FACS is to identify and sort for specific mutants within a population or select for positive transfected cells. Use of appropriate antibodies to detect the product of the transfected gene or use of plasmids that generate fluorescent products allows for ready identification of the transfected cells. The

current instrument will sort cells at a rate of 5,000-7,000 cells/sec and could be upgraded with TURBOSORT to allow sorting of 15,000 cells/sec at greater than 98% purity.

(v) Pharmaceutical Preparations

While it is possible for secretin therapeutics, such as secretin agonists or cellular compositions, to be administered as pure or substantially pure compounds/compositions, it is preferable that they be administered as pharmaceutical formulations or preparations. The formulations to be used in the present invention, for both humans and animals, include secretin therapeutics such as secretin agonists, or prodrug forms thereof, or cellular compositions together with one or more pharmaceutically acceptable carriers therefor, preferably a non-pyrogenic carrier, and optionally other therapeutic ingredients. A prodrug form of a secretin therapeutic or secretin agonist is a compound which, under physiological conditions, e.g., in the stomach, in the bloodstream, etc., undergoes a rearrangement, transformation, or reaction that results in a compound having increased secretin therapeutic or secretin agonist activity.

The carrier must be "acceptable" in the sense of being compatible with the active ingredient(s) of the formulation (and preferably, capable of stabilizing peptides) and not deleterious to the subject to be treated.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active ingredient(s) into association with the carrier which constitutes one or more accessory ingredients.

The present invention relates to pharmaceutical compositions of secretin therapeutics, such as secretin agonists or cellular compositions, and their uses in treating and/or preventing disorders marked by aberrant glucose metabolism, including glucose storage. In particular embodiments, the compositions of the subject methods are useful as insulinotropic agents, or to potentiate the insulinotropic effects of such molecules as GLP-1. In this regard, the present method can be useful for the treatment and/or prophylaxis of a variety of disorders, including one or more of: hyperlipemia, hyperglycemia, obesity, glucose tolerance insufficiency, insulin resistance and diabetic complications.

Secretin therapeutics, such as secretin agonists or cellular compositions can be administered in various forms, depending on the disorder to be treated and the age,

condition and body weight of the patient, as is well known in the art. For example, where the compounds are to be administered orally, they may be formulated as tablets, capsules, granules, powders or syrups; or for parenteral administration, they may be formulated as injections (intravenous, intramuscular or subcutaneous), drop infusion preparations or suppositories. For application by the ophthalmic mucous membrane route, they may be formulated as eyedrops or eye ointments. These formulations can be prepared by conventional means, and, if desired, the active ingredient may be mixed with any conventional additive, such as an excipient, a binder, a disintegrating agent, a lubricant, a corrigent, a solubilizing agent, a suspension aid, an emulsifying agent or a coating agent. Although the dosage will vary depending on the symptoms, age and body weight of the patient, the nature and severity of the disorder to be treated or prevented, the route of administration and the form of the drug, in general, a daily dosage of from 0.01 to 2000 mg of the compound is recommended for an adult human patient, and this may be administered in a single dose or in divided doses.

The phrase "therapeutically effective amount" as used herein means that amount of, e.g., a secretin therapeutic, which is effective for producing some desired therapeutic effect by enhancing, for example, the glucose responsiveness of pancreatic β cells at a reasonable benefit/risk ratio applicable to any medical treatment.

The phrase "pharmaceutically acceptable" is employed herein to refer to those secretin therapeutics, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject chemical from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose

acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

The term "pharmaceutically acceptable salts" refers to the relatively non-toxic, inorganic and organic acid addition salts of secretin therapeutics. These salts can be prepared *in situ* during the final isolation and purification of the secretin therapeutic, or by separately reacting a purified secretin therapeutic in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, napthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. (See, e.g., Berge et al. (1977) *J. Pharm. Sci.* 66:1-19)

In other cases, the secretin therapeutic useful in the methods of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable bases. The term "pharmaceutically acceptable salts" in these instances refers to the relatively non-toxic, inorganic and organic base addition salts of a secretin therapeutic. These salts can likewise be prepared *in situ* during the final isolation and purification of the secretin therapeutic, or by separately reacting the purified secretin therapeutic in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically acceptable metal cation, with ammonia, or with a pharmaceutically acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like (see, e.g., Berge et al., *supra*).

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Formulations useful in the methods of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal, aerosol and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 1 percent to about ninety-nine percent of active ingredient, preferably from about 5 percent to about 70 percent, most preferably from about 10 percent to about 30 percent.

Methods of preparing these formulations or compositions include the step of bringing into association a secretin therapeutic with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a secretin therapeutic with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia)

and/or as mouth washes and the like, each containing a predetermined amount of a secretin therapeutic as an active ingredient. A compound may also be administered as a bolus, electuary or paste.

In solid dosage forms for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, acetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered peptide or peptidomimetic moistened with an inert liquid diluent.

Tablets, and other solid dosage forms, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres.

They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active secretin therapeutic, such as a secretin agonist or cellular composition, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more secretin therapeutics such as secretin agonists with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active agent.

Formulations which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of a secretin therapeutic include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active component may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to secretin therapeutics, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to a secretin therapeutic, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Secretin therapeutics, such as a secretin agonist, can be alternatively administered by aerosol. This is accomplished by preparing an aqueous aerosol, liposomal preparation or solid particles containing the compound. A nonaqueous (e.g., fluorocarbon propellant) suspension could be used. Sonic nebulizers are preferred because they minimize exposing the agent to shear, which can result in degradation of the compound.

Ordinarily, an aqueous aerosol is made by formulating an aqueous solution or suspension of the agent together with conventional pharmaceutically acceptable carriers and stabilizers. The carriers and stabilizers vary with the requirements of the particular compound, but typically include nonionic surfactants (Tweens, Pluronics, or polyethylene glycol), innocuous proteins like serum albumin, sorbitan esters, oleic acid, lecithin, amino acids such as glycine, buffers, salts, sugars or sugar alcohols. Aerosols generally are prepared from isotonic solutions.

Transdermal patches have the added advantage of providing controlled delivery of a secretin therapeutic such as a secretin agonist to the body. Such dosage forms can be made by dissolving or dispersing the agent in the proper medium. Absorption enhancers can also

be used to increase the flux of the peptidomimetic across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the peptidomimetic in a polymer matrix or gel.

Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention.

Pharmaceutical compositions of this invention suitable for parenteral administration comprise a secretin therapeutic, such as a secretin agonist, in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively,

delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of a secretin therapeutic, such as a secretin agonist, or in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

When a secretin therapeutic, such as a secretin agonist or cellular composition, is administered as a pharmaceutical, to humans and animals, it can be given *per se* or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

The preparations of agents may be given orally, parenterally, topically, or rectally. They are of course given by forms suitable for each administration route. For example, they are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, etc. administration by injection, infusion or inhalation; topical by lotion or ointment; and rectal by suppositories. Oral administration is preferred.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, infraorbital, intra cardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The phrases "systemic administration," "administered systemically," "peripheral administration" and "administered peripherally" as used herein mean the administration of a secretin therapeutic, such as a secretin agonist, drug, or other material other than directly into the central nervous system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

Secretin therapeutics such as a secretin agonist may be administered to humans and other animals for therapy by any suitable route of administration, including orally, nasally,

as by, for example, a spray, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually.

Regardless of the route of administration selected, a secretin therapeutic, such as a secretin agonist, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

(vi) Conjoint Administration

Another aspect of the invention provides a conjoint therapy wherein one or more other therapeutic agents are administered with a secretin therapeutic, such as a secretin agonist or cellular compositions. Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate dosing of the individual components of the treatment.

In one embodiment, a secretin therapeutic, such as a secretin agonist or cellular compositions, may be administered alone or in combination with other agents that augment the biological activity of secretin, the biological effect of secretin, or to lessen any possible side-effects. For example, secretin or a secretin agonist may be administered together with an agent that acts as an inhibitor of the insulin inhibition of secretin bioactivity. Thus, secretin or its analog or mimetic may be conjointly administered with such an agent.

In another illustrative embodiment, a secretin therapeutic, such as a secretin agonist or cellular compositions, can be conjointly administered with an M1 receptor antagonist. Cholinergic agents are potent modulators of insulin release that act via muscarinic receptors. Moreover, the use of such agents can have the added benefit of decreasing cholesterol levels, while increasing HDL levels. Suitable muscarinic receptor antagonists include substances that directly or indirectly block activation of muscarinic cholinergic receptors. Preferably, such substances are selective (or are used in amounts that promote such selectivity) for the M1 receptor. Nonlimiting examples include quaternary amines (e.g., methantheline, ipratropium, and propantheline), tertiary amines (e.g., as dicyclomine, scopolamine) and tricyclic amines (e.g., telenzepine). Pirenzepine and methyl scopolamine

are preferred. Other suitable muscarinic receptor antagonists include benztropine (commercially available as COGENTIN from Merck), hexahydro-sila-difenidol hydrochloride (HHSID hydrochloride disclosed in Lambrecht, et al. (1989) *Trends in Pharmacol. Sci.* 10(Suppl):60; (+/-)-3-quinuclidinyl xanthene-9-carboxylate hemioxalate (QNX-hemioxalate; Birdsall, et al. (1983) *Trends in Pharmacol. Sci.* 4:459; telenzepine dihydrochloride (Coruzzi, et al. (1989) *Arch. Int. Pharmacodyn. Ther.* 302:232; and Kawashima, et al. (1990) *Gen. Pharmacol.* 21:17) and atropine. The dosages of such muscarinic receptor antagonists will be generally subject to optimization as outlined above. In the case of lipid metabolism disorders, dosage optimization may be necessary independently of whether administration is timed by reference to the lipid metabolism responsiveness window or not.

A secretin therapeutic, such as a secretin agonist or cellular compositions used according to the invention can also be used conjointly with agents acting on the ATP-dependent potassium channel of the β-cells, such as glibenclamide, glipizide, gliclazide and AG-EE 623 ZW. Secretin or its analog or mimetic may also advantageously be applied in combination with other oral agents such as metformin and related compounds or glucosidase inhibitors as, for example, acarbose.

In yet another embodiment, a secretin therapeutic may be administered together with a pyy protein or therapeutic.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, *Molecular Cloning A Laboratory Manual*, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press:1989); *DNA Cloning*, Volumes I and II (D. N. Glover ed., 1985); *Oligonucleotide Synthesis* (M. J. Gait ed., 1984); Mullis *et al.* U.S. Patent No. 4,683,195; *Nucleic Acid Hybridization* (B. D. Hames & S. J. Higgins eds. 1984); *Transcription And Translation* (B. D. Hames & S. J. Higgins eds. 1984); *Culture Of Animal Cells* (R. I. Freshney, Alan R. Liss, Inc., 1987); *Immobilized Cells And Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide To Molecular Cloning* (1984); the treatise, *Methods In Enzymology* (Academic Press, Inc., N.Y.); *Gene Transfer Vectors For Mammalian Cells* (J. H. Miller and M. P. Calos eds., 1987, Cold

Spring Harbor Laboratory); *Methods In Enzymology*, Vols. 154 and 155 (Wu et al. eds.), *Immunochemical Methods In Cell And Molecular Biology* (Mayer and Walker, eds., Academic Press, London, 1987); *Handbook Of Experimental Immunology*, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986).

All of the above-cited references and publications are incorporated herein by reference.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

Examples

Materials and Methods

A: Method of Isolation

These conditions are optimized for pancreas from rats aged 1 week to old adult. Small ducts from rat pancreas are isolated after enzymatic digestion in Collagenase A (Boehringer-Mannheim). Alternatively, Collagenase H will also work. The collagenase is dissolved in DMEM/F12 at 1U/ml. Digestion is at 37 °C for 40 min in a shaking water bath. The digest is vortexed (to further aid dissociation) and washed by centrifugation at 1000 rpm for 5 min. The pellet is resuspended in HBSS (Ca/Mg free) and poured through a 500 μm mesh to remove large particles and washed again. For older animals (over 2 weeks) the digest is resuspended in HBSS and placed in a 100 mm plate. The floating duct fragments are isolated manually with a pipette. For larger yields, pancreas from 2 week rat pups can be separated on Percoll (Pharmacia). In older pups the density of the exocrine tissue becomes the same as the density of the ducts and separation on percoll may not work.

The digested pancreas from the end of step one is overlaid on a 40% Percoll solution and centrifuged at 1900 rpm for 10 min. The duct fragments are located at the interface of buffer and Percoll at the top of the tube. This material is washed and placed in a 100 mm dish. Contaminating islets (very few) are removed manually. The fragments (including single cells) are washed again and plated for culture. These methods have been verified to give very low levels of contaminating insulin positive cells. See Figures 1 and 2.

B: Method of Culture

Duct fragments are preferentially cultured in Iscoves modified DMEM with 5% FBS and penicillin/streptomycin. DMEM and DMEM/F12 are also suitable media for culture. In the ideal embodiment, culture for 5 days will achieve a confluent monolayer that can then be induced to differentiate. Confluence of the entire monolayer is not essential; differentiation can take place on any patch of confluent cells. See Figures 3 and 4.

The monolayer can be grown in the presence of EGF (10 ng/ml) or TGF-α (10 ng/ml) to enhance growth. Induction of differentiation is believed to be cAMP dependent. Agents which induce an increase in intracellular cAMP levels are anticipated to induce differentiation. Dexamethasone, cholera toxin, forskolin, dibutyryl cAMP and Na-butyrate have all been tested and found to induce differentiation. See Figure 5.

Induction of differentiation is preferentially done in a single treatment for 48 hr. Progenitor cells appear over the course of the 48 hr treatment. Treatment can also be done for 24 hr resulting in progenitor cells. See Figure 6.

Substantially pure pancreatic ducts were placed into monolayer cultures in the presence of DMEM and 5% FCS, Pen-Strep, and Glutamine (1 mM). All culture components were ordered from Gibco-BRL. After five days, the cultures were treated with compounds as indicated (Sigma). After incubation for 48 hours, non-adherent cells (NACs) were counted and collected for insulin content analysis. After pelleting, the cells were extracted into acid:ethanol and sonicated. The resulting extract was then dried down and resuspended in 10 mM HCl and neutralized into PBS, 0.1% Triton X-100, 0.1% BSA (Sigma, Fraction V). Dilutions were then assayed in an insulin ELISA (Crystal Diagnostics). Results of these assays are displayed in Figures 7-10.

We Claim:

1. A method for modulating the growth state of pancreatic cells comprising contacting the cells with a secretin therapeutic or prodrug form thereof.

2. A method for promoting proliferation of pancreatic cells comprising contacting the cells with a secretin therapeutic or prodrug form thereof.

3. The method of claim 1 or 2, wherein the secretin therapeutic is provided as part of a composition also comprising a pharmaceutically acceptable excipient.

4. The method of claim 1 or 2, wherein the method is performed *in vitro*.

5. The method of claim 1 or 2, wherein the pancreatic cells comprise pancreatic tissue.

6. The method of claim 1 or 2, wherein the pancreatic cells include epithelial cells.

7. The method of claim 1 or 2, wherein the pancreatic cells include ductal epithelial cells.

8. The method of claim 1 or 2, wherein the secretin therapeutic comprises a polypeptide sequence at least 60% identical to SEQ ID No. 2 or an active fragment thereof.

9. The method of claim 1 or 2, wherein the secretin therapeutic comprises a secretin peptidomimetic.

10. The method of claim 9, wherein the secretin peptidomimetic is a derivative of a polypeptide at least 60% identical to SEQ ID No. 2 wherein one or more amide bonds is replaced with a protease-resistant bond, whereby the peptidomimetic has a serum half-life longer than the peptide represented in SEQ ID No. 2.

11. The method of claim 1 or 2, wherein the secretin therapeutic comprises a non-peptidyl secretin agonist.

12. The method of claim 11, wherein the secretin agonist is a small organic molecule.

13. A method for modifying glucose metabolism in an animal, comprising administering to the animal a pharmaceutically effective amount of a secretin therapeutic.

14. A method for treating a disease associated with altered glucose metabolism, comprising administering to an animal a pharmaceutically effective amount of a secretin therapeutic.

15. A method for treating a disease associated with altered glucose metabolism, comprising administering to an animal a secretin therapeutic and a pharmaceutically effective amount of a composition comprising secretin-responsive islets or cells, wherein the therapeutic and the islets or cells are administered either simultaneously or sequentially.

16. The method of claim 14 or 15, wherein said disease is associated with a condition selected from the group consisting of insulin resistance, glucose intolerance or glucose non-responsiveness, hyperglycemia, hyperinsulinemia, obesity, hyperlipidemia and hyperlipoproteinemia in a subject.

17. The method of claim 16, wherein said disease is Type II diabetes mellitus (NIDD).

18. A method for generating a functional pancreatic β cell, comprising contacting a pancreatic islet or cell with a secretin therapeutic.

19. The method of claim 18, wherein the pancreatic islet or cell is contacted with a

secretin therapeutic *in vitro*.

20. The method of claim 14, 15, 16, or 18, wherein the secretin therapeutic is provided as part of a composition also comprising a pharmaceutically acceptable excipient.

21. The method of claim 14, 15, 16, or 18, wherein said secretin therapeutic promotes insulin production in a pancreatic islet or cell.

22. The method of claim 14, 15, 16, or 18, wherein said secretin therapeutic antagonizes insulin inhibition of secretin response in secretin-responsive cells.

23. The method of claim 14, 15, 16, or 18, wherein said secretin therapeutic comprises an agent capable of inhibiting the degradation of secretin or a secretin agonist.

24. The method of claim 14, 15, 16, or 18, wherein said secretin therapeutic binds to a secretin-responsive receptor.

25. The method of claim 14, 15, 16, or 18, wherein said secretin therapeutic is vector including a nucleic acid encoding a polypeptide at least 60% identical to SEQ ID No. 2 or a biologically active fragment thereof.

26. The method of claim 14, 15, 16, or 18, wherein said secretin therapeutic is a small organic molecule.

27. The method of claim 14, 15, or 16, further comprising administering an agent capable of inhibiting the degradation of secretin or a secretin agonist.

28. The method of claim 27, wherein said agent is administered simultaneously with secretin or a secretin agonist.

29. The method of claim 14, 15, or 16, further comprising administering to the animal insulin, a dipeptidylpeptidase inhibitor, or GLP-1.

30. A method for isolating secretin-responsive cells, comprising
providing a sample of cells,
identifying in the sample cells responsive to secretin, and
isolating the identified cells from the sample of cells.

31. The method of claim 30, wherein the sample of cells comprises pancreatic cells.

32. The method of claim 30, wherein the sample of cells comprises pancreatic ductal epithelial cells.

33. The method of claim 30, wherein identifying cells responsive to secretin comprises contacting the sample of cells with secretin or a secretin agonist and identifying cells characterized by an increased rate of proliferation in the presence of the secretin or the secretin agonist.

34. The method of claim 30, wherein identifying cells responsive to secretin comprises contacting the sample of cells with secretin or a secretin agonist and identifying cells characterized by an increased concentration of insulin in the presence of the secretin or the secretin agonist.

35. The method of claim 30, wherein identifying cells responsive to secretin comprises contacting the sample of cells with secretin or a secretin agonist and identifying cells characterized by an altered growth state in the presence of the secretin or the secretin agonist.

36. The method of claim 30, wherein isolating the identified cells includes separating the cells using an automatic cell sorter.

37. The method of claim 30, wherein identifying cells responsive to secretin

comprises contacting the sample of cells with an antibody immunoreactive with a secretin-responsive receptor and identifying cells bound by the antibody.

38. The method of claim 30, wherein identifying cells responsive to secretin comprises contacting the sample of cells with a detectably labelled derivative of secretin and identifying cells bound by the detectably labelled derivative of secretin.

39. A substantially pure sample of cells isolated by the method of claim 30.

40. A substantially pure sample of cells, wherein the cells are pancreatic progenitor cells which respond to secretin by either increasing insulin production or by proliferating at an increased rate.

41. A composition comprising cells of claim 39 or 40 and a pharmaceutically acceptable carrier or a biocompatible polymer.

42. A method for identifying a secretin therapeutic, comprising

providing a sample of cells,

treating the sample of cells with a test agent, and

comparing the sample of cells to an control sample of cells in the absence of the test agent,

wherein an increase in the insulin concentration, the insulin expression, the rate of proliferation, or the rate of differentiation of the treated cells, relative to the untreated cells, is indicative of secretin therapeutic activity.

43. The method of claim 42, wherein the sample of cells comprises pancreatic cells.

44. The method of claim 42, wherein the sample of cells comprises insulin-positive progenitor cells.

45. A secretin therapeutic identified by the method of claim 42.

46. A method for identifying a secretin antagonist, comprising
providing a sample of cells,
treating the sample of cells with a test agent,
treating the sample of cells with secretin or a secretin agonist, and
comparing the sample of cells to a control sample of cells treated with secretin
in the absence of the test agent,
wherein an increase in the insulin concentration, the insulin expression, the rate of proliferation, or the rate of differentiation of the control cells, relative to the treated cells, is indicative of secretin antagonist activity.

47. The method of claim 46, wherein the sample of cells comprises pancreatic cells.

48. The method of claim 46, wherein the sample of cells comprises insulin-positive progenitor cells.

49. A compound identified by the method of claim 46.

50. A method for identifying an agent which antagonizes insulin inhibition of secretin response, comprising
providing a sample of cells,
treating the sample of cells with insulin,
treating the sample of cells with secretin or a secretin agonist,
treating the sample of cells with a test agent, and
comparing the sample of cells with a control sample of cells,
wherein an increase in the insulin concentration, the insulin expression, the rate of proliferation, or the rate of differentiation of the treated cells, relative to the control sample of cells, is indicative of an agent which antagonizes insulin inhibition of secretin response.

51. The method of claim 50, wherein the control sample of cells is treated with

secretin and insulin in the absence of the test agent.

52. The method of claim 50, wherein the control sample of cells is maintained in the absence of secretin.

53. The method of claim 50, wherein the sample of cells comprises pancreatic cells.

54. The method of claim 50, wherein the sample of cells comprises insulin-positive progenitor cells.

55. A compound identified by the method of claim 50.

56. A composition comprising a compound of claim 45, 49, or 55 and a pharmaceutically acceptable excipient.

57. The composition of claim 56, further comprising a substantially pure sample of cells which respond to secretin by either increasing insulin production or by proliferating at an increased rate.

58. The composition of claim 57, wherein the cells are pancreatic cells.

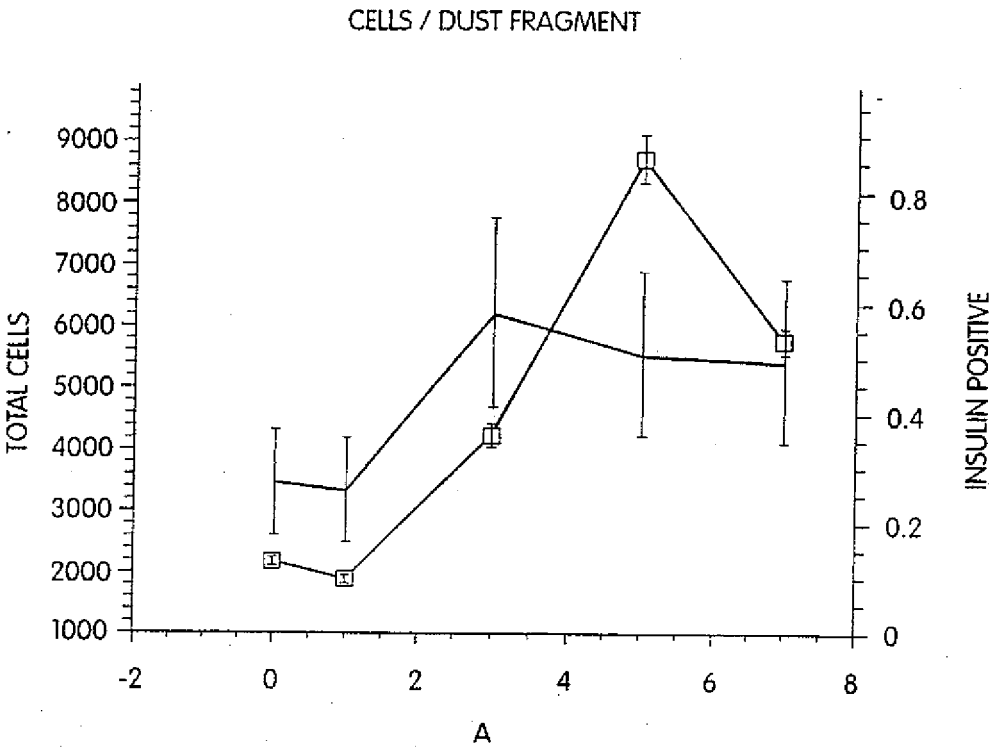

CELLS / DUST FRAGMENT
TOTAL CELLS
9000
8000
7000
6000
5000
4000
3000
2000
1000
INSULIN POSITIVE
0.8
0.6
0.4
0.2
0
-2
0
2
4
6
8
A

Fig. 1

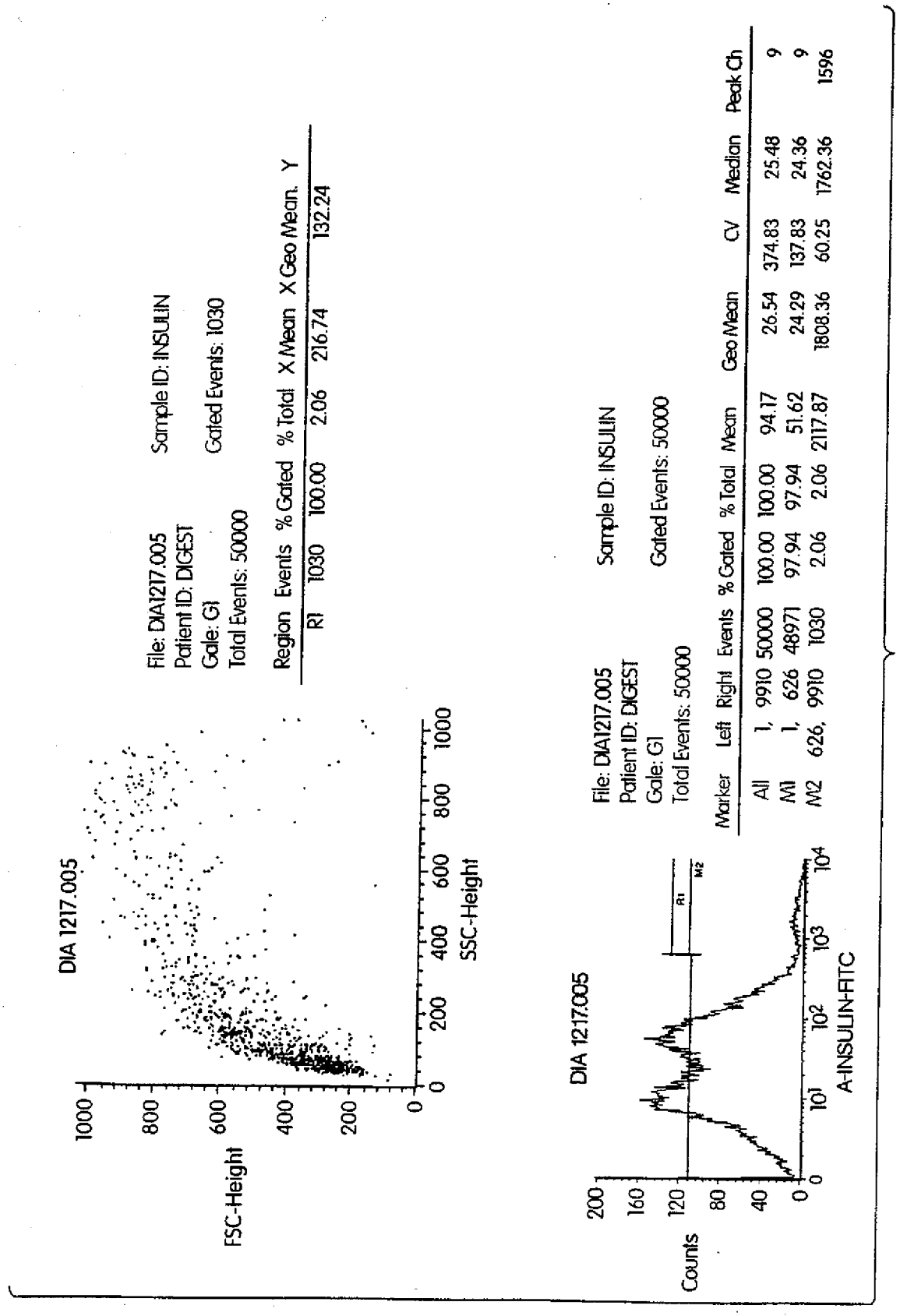

DIA 1217.005
File: DIA1217.005
Patient ID: DIGEST
Gate: G1
Total Events: 50000
Sample ID: INSULIN
Gated Events: 1030
Region Events % Gated % Total X Mean X Geo Mean Y
R1 1030 100.00 2.06 216.74 132.24
FSC-Height
SSC-Height
DIA 1217.005
File: DIA1217.005
Patient ID: DIGEST
Gate: G1
Total Events: 50000
Sample ID: INSULIN
Gated Events: 50000
Marker Left Right Events % Gated % Total Mean Geo Mean CV Median Peak Ch
All 1, 9910 50000 100.00 100.00 94.17 26.54 374.83 25.48 9
M1 1, 626 48971 97.94 97.94 51.62 24.29 137.83 24.36 9
M2 626, 9910 1030 2.06 2.06 2117.87 1808.36 60.25 1762.36 1596
Counts
A-INSULIN-FITC

Fig. 2A

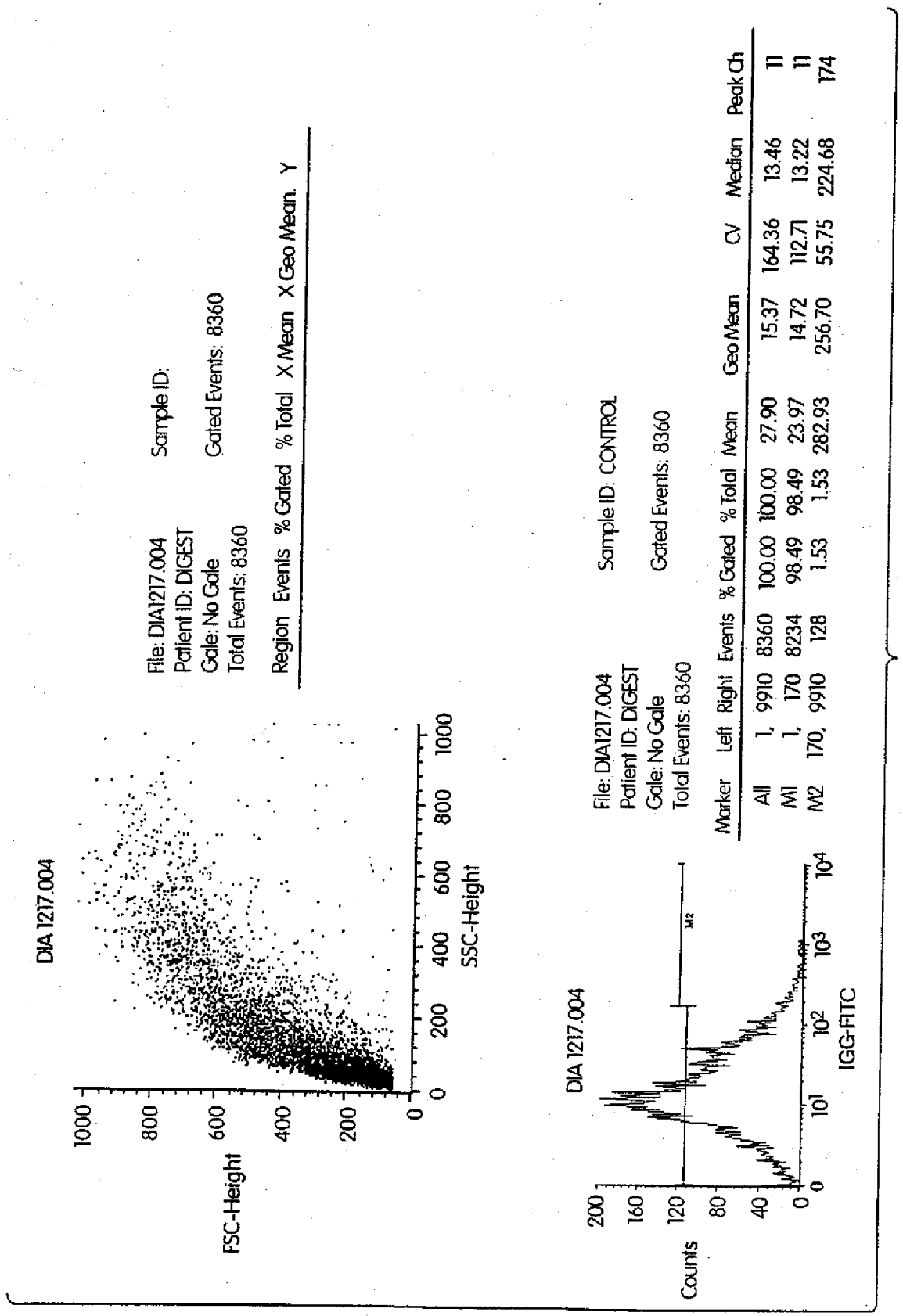

DIA 1217.004
File: DIA1217.004
Patient ID: DIGEST
Gate: No Gate
Total Events: 8360
Sample ID:
Gated Events: 8360
Region Events % Gated % Total X Mean X Geo Mean Y
FSC-Height
SSC-Height
DIA 1217.004
File: DIA1217.004
Patient ID: DIGEST
Gate: No Gate
Total Events: 8360
Sample ID: CONTROL
Gated Events: 8360
Marker Left Right Events % Gated % Total Mean Geo Mean CV Median Peak Ch
All 1, 9910 8360 100.00 100.00 27.90 15.37 164.36 13.46 11
M1 1, 170 8234 98.49 98.49 23.97 14.72 112.71 13.22 11
M2 170, 9910 128 1.53 1.53 282.93 256.70 55.75 224.68 174
Counts
IGG-FITC

Fig. 2B

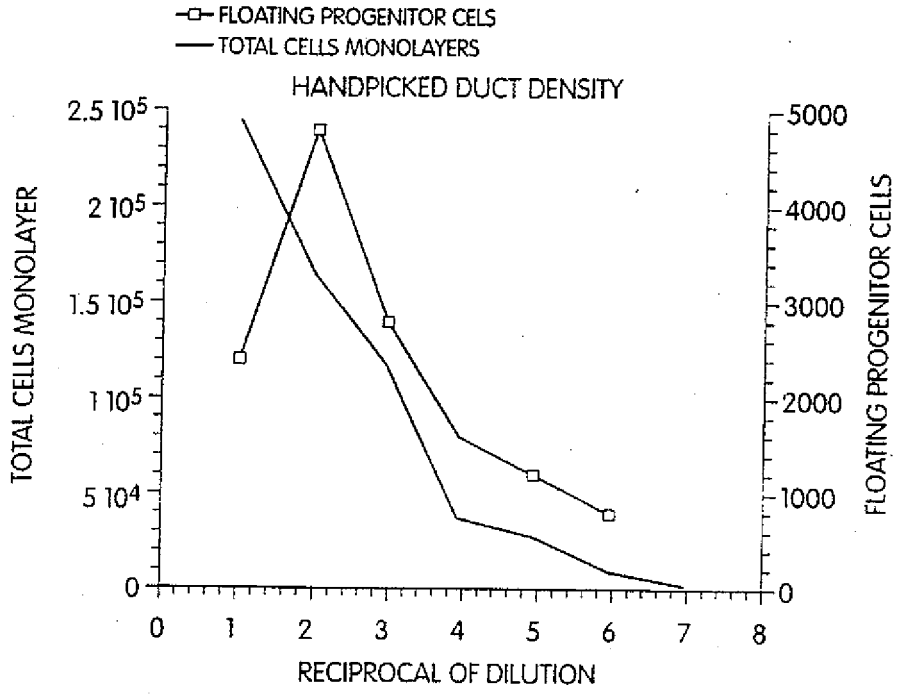

FLOATING PROGENITOR CELS
TOTAL CELLS MONOLAYERS
HANDPICKED DUCT DENSITY
TOTAL CELLS MONOLAYER
2.5 10^5
2 10^5
1.5 10^5
1 10^5
5 10^4
0
FLOATING PROGENITOR CELLS
5000
4000
3000
2000
1000
0
0 1 2 3 4 5 6 7 8
RECIPROCAL OF DILUTION

Fig. 3

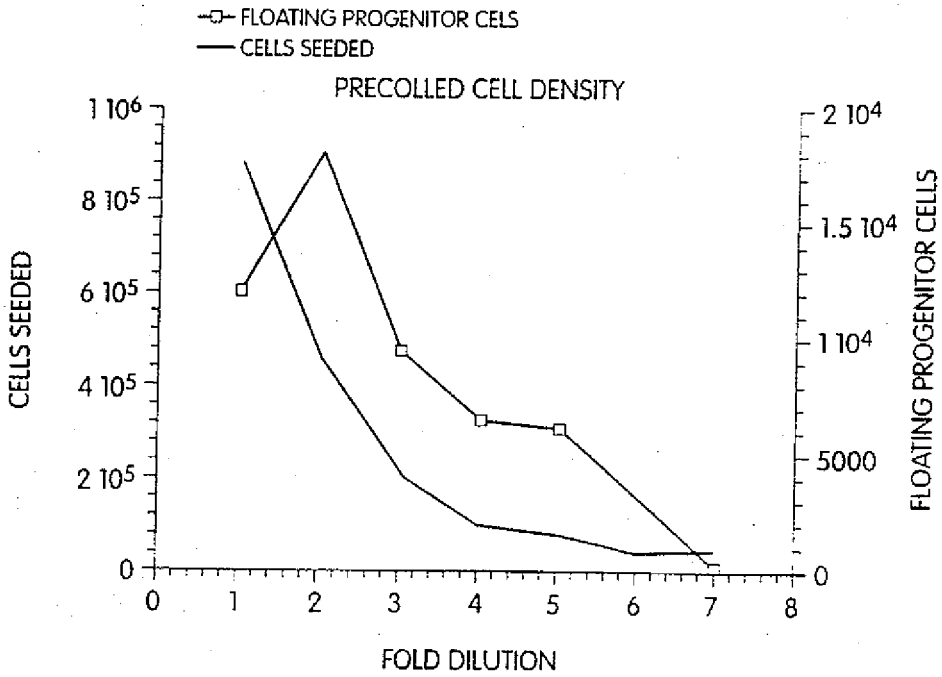

FLOATING PROGENITOR CELS
CELLS SEEDED
PRECOLLED CELL DENSITY
CELLS SEEDED
1 10^6
8 10^5
6 10^5
4 10^5
2 10^5
0
FLOATING PROGENITOR CELLS
2 10^4
1.5 10^4
1 10^4
5000
0
0 1 2 3 4 5 6 7 8
FOLD DILUTION

Fig. 4

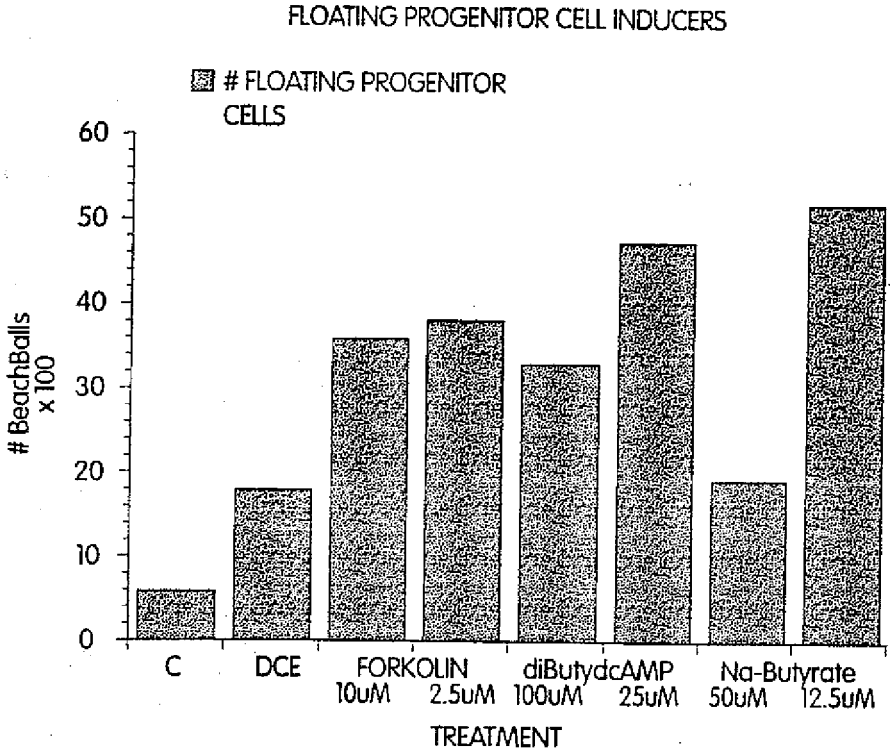

FLOATING PROGENITOR CELL INDUCERS
FLOATING PROGENITOR CELLS
BeachBalls x 100
60
50
40
30
20
10
0
C
DCE
FORKOLIN 10uM
2.5uM
diButydcAMP 100uM
25uM
Na-Butyrate 50uM
12.5uM
TREATMENT

Fig. 5

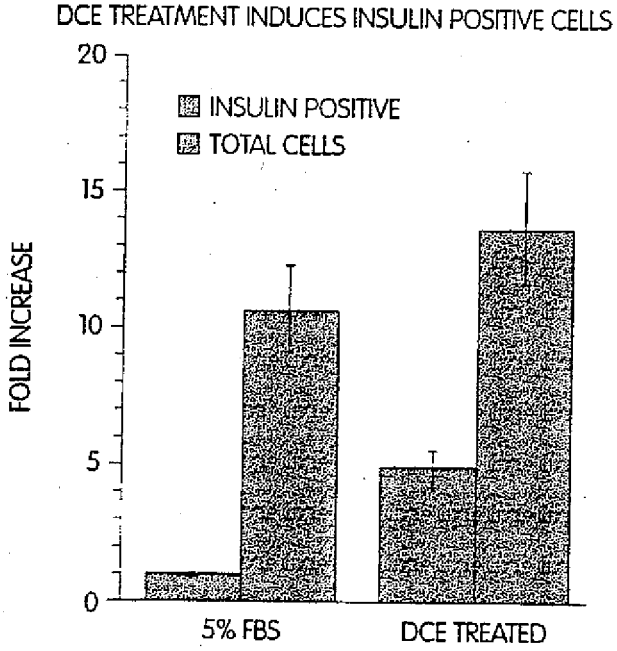

DCE TREATMENT INDUCES INSULIN POSITIVE CELLS
INSULIN POSITIVE
TOTAL CELLS
FOLD INCREASE
20
15
10
5
0
5% FBS
DCE TREATED

Fig. 6

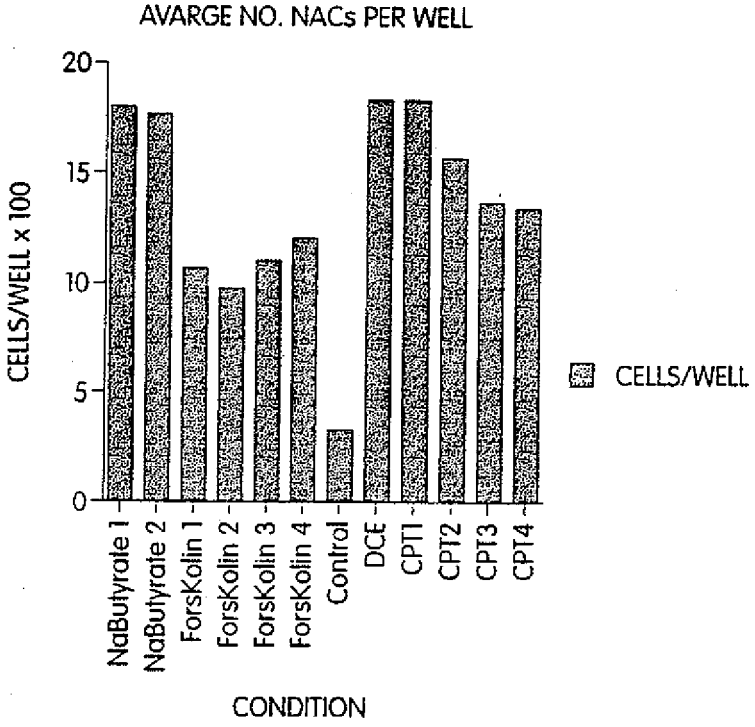

AVARGE NO. NACs PER WELL
CELLS/WELL x 100
20
15
10
5
0
NaButyrate 1
NaButyrate 2
ForsKolin 1
ForsKolin 2
ForsKolin 3
ForsKolin 4
Control
DCE
CPT1
CPT2
CPT3
CPT4
CELLS/WELL
CONDITION

Fig. 7

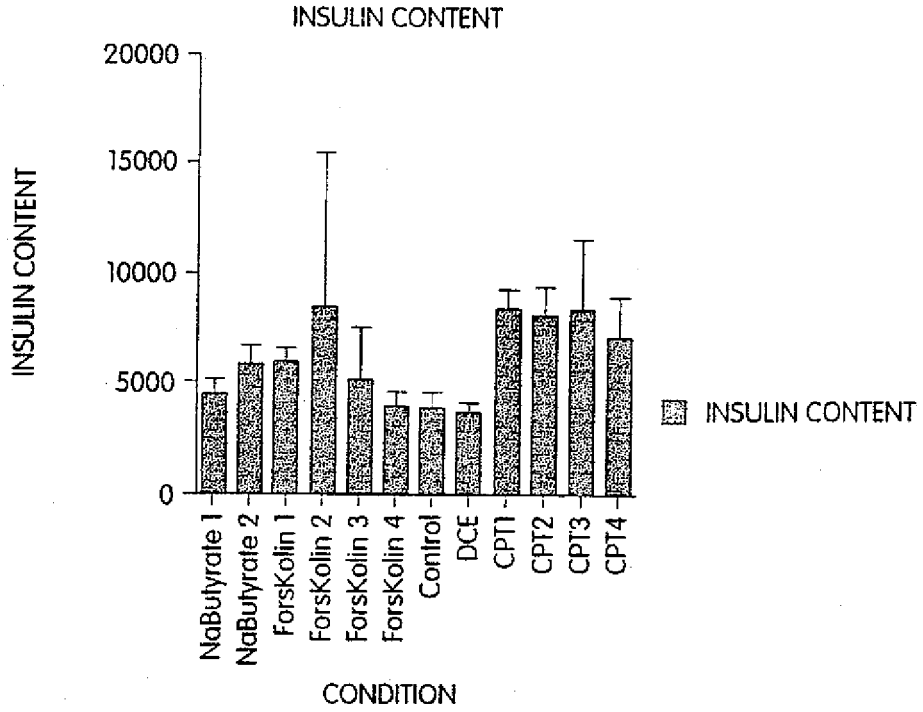

INSULIN CONTENT
INSULIN CONTENT
20000
15000
10000
5000
0
NaButyrate 1
NaButyrate 2
ForsKolin 1
ForsKolin 2
ForsKolin 3
ForsKolin 4
Control
DCE
CPT1
CPT2
CPT3
CPT4
INSULIN CONTENT
CONDITION

Fig. 8

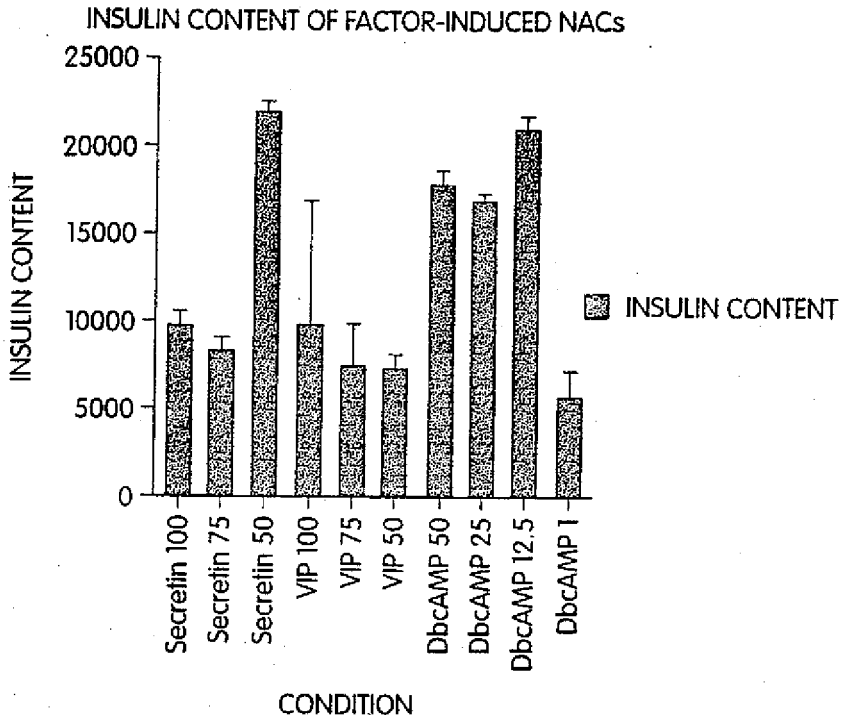

INSULIN CONTENT OF FACTOR-INDUCED NACs
INSULIN CONTENT
25000
20000
15000
10000
5000
0
Secretin 100
Secretin 75
Secretin 50
VIP 100
VIP 75
VIP 50
DbcAMP 50
DbcAMP 25
DbcAMP 12.5
DbcAMP 1
INSULIN CONTENT
CONDITION

Fig. 9

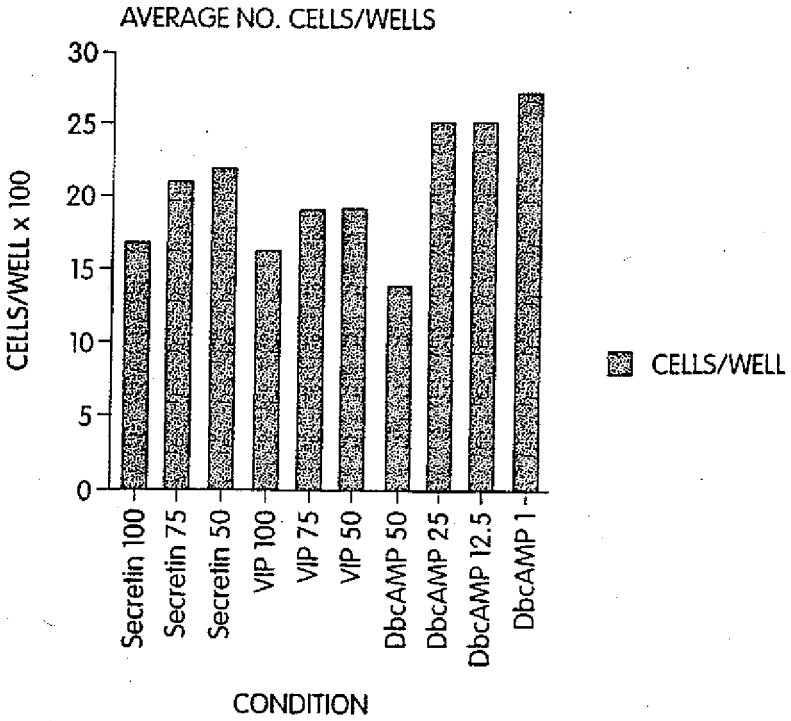

AVERAGE NO. CELLS/WELLS
CELLS/WELL x 100
30
25
20
15
10
5
0
Secretin 100
Secretin 75
Secretin 50
VIP 100
VIP 75
VIP 50
DbcAMP 50
DbcAMP 25
DbcAMP 12.5
DbcAMP 1
CELLS/WELL
CONDITION

Fig. 10

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS: Kagan, David; Pang, Kevin

(ii) TITLE OF INVENTION: Methods for Inducing Insulin Positive Progenitor Cells

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Foley, Hoag, & Eliot, LLP
(B) STREET: One Post Office Square
(C) CITY: Boston
(D) STATE: Massachusetts
(E) COUNTRY: USA
(F) ZIP: 02109

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: WordPad

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 10-FEBRUARY-1999

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: HALSTEAD, DAVID
(B) REGISTRATION NUMBER: 44,735
(C) REFERENCE/DOCKET NUMBER: ONV-063.01

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617)832-1000
(B) TELEFAX: (617)832-7000

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CAC TCA GAT GGT ACT TTC ACC TCA GAA CTA TCT CGT CTA CGT GAA GGT 48
His Ser Asp Gly Thr Phe Thr Ser Glu Leu Ser Arg Leu Arg Glu Gly

```
  1               5                  10                  15

GCA CGC CTC CAG CGC TTG CTG CAA GGT CTC GTT                          81
Ala Arg Leu Gln Arg Leu Leu Gln Gly Leu Val
             20                  25
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
His Ser Asp Gly Thr Phe Thr Ser Glu Leu Ser Arg Leu Arg Glu Gly
  1               5                  10                  15

Ala Arg Leu Gln Arg Leu Leu Gln Gly Leu Val
             20                  25
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1650 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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GGGCAGAGGG CACGGGCAGG CGGACGTCGG GGCGCCCTCG GGGAACGTGC GGGCACC ATG     60
                                                                Met
                                                                 1

CGT CCC CAC CTG TCG CCG CCG CTG CAG CAG CTA CTA CTG CCG GTG CTG CTC   111
Arg Pro His Leu Ser Pro Pro Leu Gln Gln Leu Leu Leu Pro Val Leu Leu
              5                  10                  15

GCC TGC GCC GCG CAC TCG ACT GGA GCC CTT CCC CGA CTA TGT GAC GTG CTA   162
Ala Cys Ala Ala His Ser Thr Gly Ala Leu Pro Arg Leu Cys Asp Val Leu
     20                  25                  30                  35

CAA GTG CTG TGG GAA GAG CAA GAC CAG TGC CTG CAG GAA CTC TCC AGA GAG   213
Gln Val Leu Trp Glu Glu Gln Asp Gln Cys Leu Gln Glu Leu Ser Arg Glu
                 40                  45                  50
```

```
CAG ACA GGA GAC CTG GGC ACG GAG CAG CCA GTG CCA GGT TGT GAG GGG ATG   264
Gln Thr Gly Asp Leu Gly Thr Glu Gln Pro Val Pro Gly Cys Glu Gly Met
          55                  60                  65

TGG GAC AAC ATA AGC TGC TGG CCC TCT TCT GTG CCG GGC CGG ATG GTG GAG   315
Trp Asp Asn Ile Ser Cys Trp Pro Ser Ser Val Pro Gly Arg Met Val Glu
70                  75                  80                  85

GTG GAA TGC CCG AGA TTC CTC CGG ATG CTC ACC AGC AGA AAT GGT TCC TTG   366
Val Glu Cys Pro Arg Phe Leu Arg Met Leu Thr Ser Arg Asn Gly Ser Leu
              90                  95                  100

TTC CGA AAC TGC ACA CAG GAT GGC TGG TCA GAA ACC TTC CCC AGG CCT AAT   417
Phe Arg Asn Cys Thr Gln Asp Gly Trp Ser Glu Thr Phe Pro Arg Pro Asn
     105                 110                 115                 120

CTG GCC TGT GCG GTT AAT GTG AAC GAC TCT TCC AAC GAG AAG CGG CAC TCC   468
Leu Ala Cys Ala Val Asn Val Asn Asp Ser Ser Asn Glu Lys Arg His Ser
                125                 130                 135

TAC CTG CTG AAG CTG AAA GTC ATG TAC ACC GTG GGC TAC AGC TCC TCC CTG   519
Tyr Leu Leu Lys Leu Lys Val Met Tyr Thr Val Gly Tyr Ser Ser Ser Leu
        140                 145                 150

GTC ATG CTC CTG GTC GCC CTT GGC ATC CTC TGT GCT TTC CGG AGG CTC CAC   570
Val Met Leu Leu Val Ala Leu Gly Ile Leu Cys Ala Phe Arg Arg Leu His
155                 160                 165                 170

TGC ACT CGC AAC TAC ATC CAC ATG CAC CTG TTC GTG TCC TTC ATC CTT CGT   621
Cys Thr Arg Asn Tyr Ile His Met His Leu Phe Val Ser Phe Ile Leu Arg
            175                 180                 185

GCC CTG TCC AAC TTC ATC AAG GAC GCC GTG CTC TTC TCC TCA GAT GAT GTC   672
Ala Leu Ser Asn Phe Ile Lys Asp Ala Val Leu Phe Ser Ser Asp Asp Val
    190                 195                 200                 205

ACC TAC TGC GAT GCC CAC AGG GCG GGC TGC AAG CTG GTC ATG GTG CTG TTC   723
Thr Tyr Cys Asp Ala His Arg Ala Gly Cys Lys Leu Val Met Val Leu Phe
                210                 215                 220

CAG TAC TGC ATC ATG GCC AAC TAC TCC TGG CTG CTG GTG GAA GGC CTC TAC   774
Gln Tyr Cys Ile Met Ala Asn Tyr Ser Trp Leu Leu Val Glu Gly Leu Tyr
        225                 230                 235

CTT CAC ACA CTC CTC GCC ATC TCC TTC TTC TCT GAA AGA AAG TAC CTC CAG   825
Leu His Thr Leu Leu Ala Ile Ser Phe Phe Ser Glu Arg Lys Tyr Leu Gln
240                 245                 250                 255

GGA TTT GTG GCA TTC GGA TGG GGT TCT CCA GCC ATT TTT GTT GCT TTG TGG   876
Gly Phe Val Ala Phe Gly Trp Gly Ser Pro Ala Ile Phe Val Ala Leu Trp
            260                 265                 270

GCT ATT GCC AGA CAC TTT CTG GAA GAT GTT GGG TGC TGG GAC ATC AAT GCC   927
Ala Ile Ala Arg His Phe Leu Glu Asp Val Gly Cys Trp Asp Ile Asn Ala
    275                 280                 285                 290
```

```
AAC GCA TCC ATC TGG TGG ATC ATT CGT GGT CCT GTG ATC CTC TCC ATC CTG   978
Asn Ala Ser Ile Trp Trp Ile Ile Arg Gly Pro Val Ile Leu Ser Ile Leu
                295                 300                 305

ATT AAT TTC ATC CTT TTC ATA AAC ATT CTA AGA ATC CTG ATG AGA AAA CTT  1029
Ile Asn Phe Ile Leu Phe Ile Asn Ile Leu Arg Ile Leu Met Arg Lys Leu
        310                 315                 320

AGA ACC CAA GAA ACA AGA GGA AAT GAA GTC AGC CAT TAT AAG CGC CTG GCC  1080
Arg Thr Gln Glu Thr Arg Gly Asn Glu Val Ser His Tyr Lys Arg Leu Ala
325                 330                 335                 340

AGG TCC ACT CTC CTG CTG ATC CCC CTC TTT GGC ATC CAC TAC ATC GTC TTC  1131
Arg Ser Thr Leu Leu Leu Ile Pro Leu Phe Gly Ile His Tyr Ile Val Phe
            345                 350                 355

GCC TTC TCC CCA GAG GAC GCT ATG GAG ATC CAG CTG TTT TTT GAA CTA GCC  1182
Ala Phe Ser Pro Glu Asp Ala Met Glu Ile Gln Leu Phe Phe Glu Leu Ala
    360                 365                 370                 375

CTT GCG TCA TTC CAG GGA CTG GTG GTG GCC GTC CTC TAC TGC TTC CTC AAC  1233
Leu Ala Ser Phe Gln Gly Leu Val Val Ala Val Leu Tyr Cys Phe Leu Asn
                380                 385                 390

GGG GAG GTG CAG CTG GAG GTT CAG AAG AAG TGG CAG CAA TGG CAC CTC CGT  1284
Gly Glu Val Gln Leu Glu Val Gln Lys Lys Trp Gln Gln Trp His Leu Arg
        395                 400                 405

GAG TTC CCA CTG CAC CCC GTG GCC TCC TTC AGC AAC AGC ACC AAG GCC AGC  1335
Glu Phe Pro Leu His Pro Val Ala Ser Phe Ser Asn Ser Thr Lys Ala Ser
410                 415                 420                 425

CAC TTG GAG CAG AGC CAG GGC ACC TGC AGG ACC AGC ATC ATC TGA          1380
His Leu Glu Gln Ser Gln Gly Thr Cys Arg Thr Ser Ile Ile
            430                 435                 440

GAGGCTGGAG CAGGGTCACC CATGGACAGA GACCAAGAGA GGTCCTGCGA AGGCTGGGCA    1440

CTGCTGTGGG ACAGCCAGTC TTCCCAGCAG ACACCCTGTG TCCTCCTTCA GCTGAAGATG    1500

CCCCTCCCCA GGCCTTGGAC TCTTCCGAAG GATGTGAGGC ACTGTGGGGC AGGACAAGGG    1560

CCTGGGATTT GGTTCGTTTG CTCTTCTGGG AAGAGAAGTT CAGGGGTCCC AGAAAGGGAC    1620

AGGGAAATAA ATGGTTGCCT TGGGATGAGA                                      1650
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 441 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Arg Pro His Leu Ser Pro Pro Leu Gln Gln Leu Leu Leu Pro Val Leu
                5                  10                  15

Leu Ala Cys Ala Ala His Ser Thr Gly Ala Leu Pro Arg Leu Cys Asp Val
            20                  25                  30                  35

Leu Gln Val Leu Trp Glu Glu Gln Asp Gln Cys Leu Gln Glu Leu Ser Arg
                        40                  45                  50

Glu Gln Thr Gly Asp Leu Gly Thr Glu Gln Pro Val Pro Gly Cys Glu Gly
                55                  60                  65

Met Trp Asp Asn Ile Ser Cys Trp Pro Ser Ser Val Pro Gly Arg Met Val
70                  75                  80                  85

Glu Val Glu Cys Pro Arg Phe Leu Arg Met Leu Thr Ser Arg Asn Gly Ser
                90                  95                  100

Leu Phe Arg Asn Cys Thr Gln Asp Gly Trp Ser Glu Thr Phe Pro Arg Pro
            105                 110                 115                 120

Asn Leu Ala Cys Ala Val Asn Val Asn Asp Ser Ser Asn Glu Lys Arg His
                        125                 130                 135

Ser Tyr Leu Leu Lys Leu Lys Val Met Tyr Thr Val Gly Tyr Ser Ser Ser
                140                 145                 150

Leu Val Met Leu Leu Val Ala Leu Gly Ile Leu Cys Ala Phe Arg Arg Leu
155                 160                 165                 170

His Cys Thr Arg Asn Tyr Ile His Met His Leu Phe Val Ser Phe Ile Leu
                175                 180                 185

Arg Ala Leu Ser Asn Phe Ile Lys Asp Ala Val Leu Phe Ser Ser Asp Asp
            190                 195                 200                 205

Val Thr Tyr Cys Asp Ala His Arg Ala Gly Cys Lys Leu Val Met Val Leu
                        210                 215                 220

Phe Gln Tyr Cys Ile Met Ala Asn Tyr Ser Trp Leu Leu Val Glu Gly Leu
                225                 230                 235

Tyr Leu His Thr Leu Leu Ala Ile Ser Phe Phe Ser Glu Arg Lys Tyr Leu
240                 245                 250                 255

Gln Gly Phe Val Ala Phe Gly Trp Gly Ser Pro Ala Ile Phe Val Ala Leu
                260                 265                 270

Trp Ala Ile Ala Arg His Phe Leu Glu Asp Val Gly Cys Trp Asp Ile Asn
            275                 280                 285                 290

Ala Asn Ala Ser Ile Trp Trp Ile Ile Arg Gly Pro Val Ile Leu Ser Ile
```

295 300 305

Leu Ile Asn Phe Ile Leu Phe Ile Asn Ile Leu Arg Ile Leu Met Arg Lys
310 315 320

Leu Arg Thr Gln Glu Thr Arg Gly Asn Glu Val Ser His Tyr Lys Arg Leu
325 330 335 340

Ala Arg Ser Thr Leu Leu Leu Ile Pro Leu Phe Gly Ile His Tyr Ile Val
345 350 355

Phe Ala Phe Ser Pro Glu Asp Ala Met Glu Ile Gln Leu Phe Phe Glu Leu
360 365 370 375

Ala Leu Ala Ser Phe Gln Gly Leu Val Val Ala Val Leu Tyr Cys Phe Leu
380 385 390

Asn Gly Glu Val Gln Leu Glu Val Gln Lys Lys Trp Gln Gln Trp His Leu
395 400 405

Arg Glu Phe Pro Leu His Pro Val Ala Ser Phe Ser Asn Ser Thr Lys Ala
410 415 420 425

Ser His Leu Glu Gln Ser Gln Gly Thr Cys Arg Thr Ser Ile Ile
430 435 440